Effects of thrips feeding on tospovirus transmission in chrysanthemum

Promotoren: Dr. R. W. Goldbach
Hoogleraar in de Virologie
Landbouwuniversiteit Wageningen

Dr. S. B. J. Menken
Hoogleraar in de Evolutiebiologie
Universiteit van Amsterdam

Co-promotoren: Dr. D. Peters
Universitair hoofddocent
Laboratorium voor Virologie,
Landbouwuniversiteit Wageningen

Dr. C. Mollema
Hoofdmedewerker stafafdeling Onderzoekstrategie
Dienst Landbouwkundig Onderzoek

Effects of thrips feeding on tospovirus transmission in chrysanthemum

Fennet van de Wetering

Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van de Landbouwuniversiteit Wageningen,
dr. C. M. Karssen,
in het openbaar te verdedigen op
woensdag 28 april 1999
des namiddags te 13.30 uur in de Aula.

The research described in this thesis was carried out at the Laboratory of Virology, Agricultural University Wageningen, the Netherlands.
This research was supported by a fellowship, No. ABI22.2704, of the Foundation of Dutch Technology (STW) of the Netherlands Organisation for Scientific Research (NWO).
Additional financial support by the Stichting "Landbouw Export Bureau 1916/1918" , and the Graduate School "Production Ecology" of the Wageningen Agricultural University is gratefully acknowledged.

Effects of thrips feeding on tospovirus transmission in chrysanthemum.
F. van de Wetering
Thesis Agricultural University Wageningen, the Netherlands
With references - With summary in Dutch
ISBN 90-5808-010-2
Subject headings: thrips / tospoviruses / chrysanthemum / plant diseases

Stellingen

1. Het voedingsgedrag van de trips vector op de plant bepaalt de efficiëntie van tospovirusoverdracht.

   dit proefschrift.

2. Gezien de strikte randvoorwaarden voor succesvolle overdracht van tospovirussen door tripsen wekt het verbazing dat TSWV toch tot één van de meest schadelijke plantenvirussen ter wereld behoort.

   dit proefschrift.

3. Het gebruik van trips-resistente planten kan leiden tot intensievere verspreiding van tospovirussen.

   dit proefschrift.

4. Overdracht van tospovirussen kan alleen plaatsvinden indien het virus de speekselklieren van de trips reeds voor het pop-stadium heeft bereikt.

   Wijkamp and Peters, 1993. Phytopathology 83: 986-991; Ohnishi *et al.*, 1998. Recent Progress in Tospoviruses and Thrips Research: 51-53; Tsuda *et al.*, 1996. *Phytopathology 86:* 1199-1203.

5. Vergeleken met honingbijen spelen tripsen een onderschikte rol in de overdracht van ilarvirussen.

   Bristow and Martin, 1999. Phytopathology 89: 124-130.

6. De veronderstelling dat open ruimten tussen planten de overdracht van plantenvirussen bevordert, berust meer op subjectieve waarnemingen in het veld dan op wiskundige analyses.

   Davies, 1976. Annals of Applied Biology 82: 489-501; Jones, 1993. Annals of Applied Biology 122: 501-518; Jones, 1994. Annals of Applied Biology 124: 45-58.

7. De invloed van CO_2 op het broeikaseffect is in kas-teelten wèl bewezen.

   Lindzen, 1997. Proceedings of the National Academy of Sciences of the United States of America 94: 8335-8342.

8. De overheid streeft naar extensivering van de landbouw, maar handelt daar niet naar omdat zij landbouwgrond opkoopt zonder de daarbij behorende productierechten.

9. Negatieve kritiek is de makkelijkste vorm van kritiek, maar tevens ook de méést demotiverende.

10. Vliegvermogen is van grote waarde voor de overleving van de trips, maar 'waardeloos' bij het uitvoeren van lab-experimenten.

11. Dat de nieuwe (niet-)buitenspelregel, die het hockey zo veel attractiever heeft gemaakt, nog niet is ingevoerd bij voetbal getuigt van een gebrek aan commercieel inzicht.

12. Het wonder van het leven is het leven zelf.

13. Aangezien alle wegen naar Rome leiden, kun je niet verdwalen wanneer je de weg van Rome naar huis weet.

Stellingen behorende bij het proefschrift

' Effects of thrips feeding on tospovirus transmission in chrysanthemum',

door Fennet van de Wetering, te verdedigen op woensdag 28 april 1999 te Wageningen.

Contents

Chapter 1

General introduction

During the last two decades there has been a marked increase of outbreaks of tomato spotted wilt disease in a great variety of economically important vegetable and ornamental crops. The causal agent of this disease is tomato spotted wilt tospovirus (TSWV, genus *Tospovirus*) which is transmitted by thrips, a group of minute insects. To date, durable control of TSWV appears very hard, if not impossible. The broad host range of TSWV, the global transport of plant material, the unintentional spread of vectors -especially the western flower thrips (*Frankliniella occidentalis* Pergande [Thysanoptera: Thripidae])-, the easy adaptation of thrips to pesticides and the huge number of plant species on which these insects are found, are major factors which have made TSWV as one of the most devastating plant viruses world-wide. Moreover, other tospoviruses related to TSWV, have become increasingly damaging in recent years. Hence, there is a growing demand for alternative control measures for tospoviruses and their vectors, such as biological control of thrips and development and introduction of resistant crops, which can be integrated in the existing physical, cultural and chemical control measures. To realise sustainable management strategies it will be necessary to unravel the interactions between the tospovirus, its vectors and its host plants. The objective of the research, as reported in this PhD thesis, was to add to this required knowledge thereby focussing on the interactions between TSWV/*F. occidentalis*/chrysanthemum. In the Netherlands, chrysanthemum represents an economically important ornamental crop and both *F. occidentalis* (pest insect number one in greenhouses) and TSWV (most abundant tospovirus in this crop) threaten its production. Before describing the results obtained, an introduction will be given to the virus, its thrips vectors, tospovirus transmission and its current management strategies.

Biological and molecular properties of tospoviruses

TSWV was discovered in Australia in tomato in 1915 (Brittlebank, 1919). The onion thrips, *Thrips tabaci* (Lindeman) was reported to be a vector of this virus (Pittman, 1927) and believed to be the main transmitter for several decennia. Due to unknown reasons, TSWV faded away almost completely in Western Europe in the 1950s.

Table 1. Members of the genus *Tospovirus*, their geographical distribution and thrips vectors

Tospovirus species				
Nomenclature	Abbreviation	Sero-group	Geographical distribution	Vector
Tomato spotted wilt virus	TSWV[1]	I	World-wide, except for arctic regions[12]	*F. bispinosa*[22] *F. fusca*[23] *F. intonsa*[24] *F. occidentalis*[25] *F. schultzei*[24, 26] *T. setosus*[27, 28] *T. tabaci*[29]
Tomato clorotic spot virus	TCSV[2]	II	Argentine[13], Brazil[2]	*F. occidentalis*[24] *F. schultzei*[24] *F. intonsa*[24]
Groundnut ringspot virus	GRSV[2]	II	South Africa, Brazil[2], Argentine[14]	*F. occidentalis*[24] *F. schultzei*[24]
Impatiens necrotic spot virus	INSV[3]	III	USA[3], the Netherlands[15], Italy[16], France[17], Portugal[18]	*F. occidentalis*[30,31]
Groundnut bud necrosis virus	GBNV[4]	IV	India[4]	*T. palmi*[32,33,34] *F. schultzei*[32,35]
Watermelon silver mottle virus	WSMV[5]	IV	Japan[19], Taiwan[5]	*T. palmi*[36]
Watermelon bud necrosis virus	WBNV[6]	IV	India[6]	*T. flavus*/ *T. palmi?*[37]
Peanut yellow spot virus	PYSV[7]	V	India, Thailand[7]	*?*
Iris yellow spot virus	IYSV[8]	VI	Brazil[10], Israel[20], the Netherlands[8]	*T. tabaci*[38]
Physalis severe mottle virus	PSMV[9]	VII	Thailand[9]	*?*
Chrysanthemum stem necrosis virus	CSNV[10]	VIII	Brazil[10], the Netherlands[21]	*?*
Zucchini lethal chlorotic virus	ZLCV[10]	IX	Brazil[10]	*?*
Peanut chlorotic fan-spot virus	PCFV[11]	X	Taiwan[11]	*S. dorsalis*/ *T. palmi?*[39]

The renewed incidence of TSWV in this part of Europe in the 1980s has been attributed to unintentional introduction of another vector: the western flower thrips, *Frankliniella occidentalis* (Pergande) from the USA (Zur Strassen, 1986). To date, at least five other thrips species are known to vector TSWV: *F. bispinosa*, *F. fusca*, *F. intonsa*, *F. schultzei* and *T. setosus*. Also *T. palmi* has been reported to transmit TSWV (Fujisawa *et al.*, 1988), although its status as vector has, so far, not adequately been demonstrated. TSWV was long presumed to be the sole member of the *Tospovirus* genus within the family Bunyaviridae (Murphy *et al.*, 1995). In the last seven years, new tospoviruses have been reported, differing in serology, genome sequence, vector specificity and host range (Goldbach and Kuo, 1996). Currently, thirteen tospovirus species are distinguished and divided into ten serogroups (Table 1). They are transmitted by eight, possibly ten thrips species, if *Scirthothrips dorsalis* and *T. flavus* are included (see following section). Of all tospoviruses identified so far, TSWV is most frequently encountered and is spread by most of the thrips vectors.

In the Netherlands, so far, the occurrence of the tospovirus species TSWV, impatiens necrotic spot virus (INSV), iris yellow spot virus (IYSV) and chrysanthemum stem necrosis virus (CSNV) has been confirmed (Verhoeven and Roenhorst, 1998). TSWV and INSV need to be controlled, whereas IYSV and CSNV are still of minor importance, perhaps even being absent at the moment. IYSV has been found twice in bulbous crops, i.e. in 1992 in iris (Derks and Lemmers, 1996) and in 1997 in onion (Verhoeven and Roenhorst, 1998). It is assumed that this tospovirus has been introduced in Israel with infected Dutch bulbs (Gera *et al.*, 1998a), although this is not confirmed as this virus has not been found in bulbs used for export in the Netherlands. Of the four tospoviruses present in the Netherlands only two have been found infecting chrysanthemum. The first, CSNV, has been isolated from chrysanthemum in 1994 and 1995 (Verhoeven *et al.*, 1996). Infected chrysanthemum plants were only found at companies that had imported cuttings from Brazil, the only other country where this virus has been detected so far. After 1995, this tospovirus species has not been found to cause severe infections in chrysanthemum crops in the Netherlands.

Legend of Table 1:
?: vector not known; [1]de Haan, 1991; [2]de Àvila *et al.*, 1990; [3]Law and Moyer, 1990; [4]Satyanarayana *et al.*, 1996; [5]Yeh and Chang, 1995; [6]Jain *et al.*, 1998; [7]Reddy *et al.*, 1990; [8]Cortês *et al.*, 1998; [9]Cortês *et al.*, 1998; [10]Resende *et al.*, 1996; [11]Chen and Chiu, 1996; [12]Goldbach and Peters, 1994; [13]Granval de Millan *et al.*, 1998; [14]Dewey *et al.*, 1993; [15]de Àvila *et al.*, 1992; [16]Vaira *et al.*, 1993; [17]Marchoux *et al.*, 1991; [18]Louro, 1996; [19]Iwaki *et al.*, 1984; [20]Gera *et al.*, 1998a; [21]Verhoeven *et al.*, 1996; [22]Webb *et al.*, 1998; [23]Sakimura, 1963; [24]Wijkamp *et al.*, 1995; [25]Gardner *et al.*, 1935; [26]Samuel *et al.*, 1930; [27]Fujisawa *et al.*, 1988; [28]Tsuda *et al.*, 1996; [29]Pittman, 1927; [30]DeAngelis *et al.*, 1993; [31]Wijkamp and Peters, 1993; [32]Lakshmi *et al.*, 1995; [33]Palmer *et al.*, 1990; [34]Vijayalakshmi, 1994; [35]Amin *et al.*, 1981; [36]Yeh *et al.*, 1992; [37]Singh and Krishanareddy, 1996; [38]Gera *et al.*, 1998b; [39]Yeh *et al.*, 1998.

The second tospovirus occurring in Dutch chrysanthemum, TSWV, still threatens its production. Not only does it infect many greenhouse crops (Verhoeven and Roenhorst, 1998), but also adjacent outdoor-grown vegetables and weeds (Verhoeven and Roenhorst, 1994). Remarkably, INSV, a tospovirus that predominantly occurs in ornamental crops, has not been infecting chrysanthemum in the Netherlands (Verhoeven and Roenhorst, 1998).

To date, the world-wide impact of TSWV is large, as it encompasses a host range of at least 900 species including both dicotyledon and monocotyledon plant species (Peters, 1998). Symptoms of TSWV infection greatly depend on plant species, cultivars and growing conditions (Adam and Kegler, 1994), and vary from relatively mild, e.g. chlorosis, mottling, stunting and wilting, to severe, e.g. necrosis of leaf and stem tissue and even death of the plant. In chrysanthemum, TSWV causes severe cosmetic damage, with symptoms consisting of chlorotic to necrotic spots on inoculated leaves, and systemic symptoms such as leaf collapse, necrosis of veins, at times leading to plant death (Daughtrey *et al.*, 1997; Matteoni and Allen, 1989). In some chrysanthemum cultivars, however, TSWV infection remains unnoticed due to poor or no symptom expression (Matteoni and Allen, 1989).

Electron microscopical analysis demonstrated that TSWV has quasispherical, enveloped particles, ranging from 70 to 110 nm in diameter (Fig.1). Purified particles consist of three genomic RNA segments and four structural proteins, i.e. the nucleocapsid protein (N), two types of glycoproteins (G1 and G2) and the L protein, representing the putative viral polymerase. The N protein is tightly associated with the single stranded RNA segments, denoted S (small), M (medium) and L (large) RNA, of which the last-mentioned is of negative polarity whereas the S and M RNA display an ambisense gene arrangement. Two non-structural proteins, NS_s and NS_m, are encoded by the viral sense sequence of the S and M RNA. The NS_m protein represents the viral movement protein, essential for cell-to-cell spread of the virus during systemic (plant) infection (Kormelink *et al.*, 1994; Storms, 1998), whereas the function of NS_s is still unknown.

Thrips vectors of tospoviruses

Thrips are little insects, usually only a few millimetres long, and belong to the order *Thysanoptera,* a name derived from the band-link wings fringed with long cilia. Of an estimated 8000 species, only 5000 are now taxonomically described and placed into two suborders and eight families (Gaston and Mound, 1993; Mound and Kibby, 1998). Thrips identification is based on morphological characteristics, such as surface structures, presence and position of setae (hairs) on the head, thorax and abdomen. Misidentification due to inaccurate preparation of these small insects and to intraspecific variation in e.g. size and colour are easily made (Palmer *et al.*, 1990). An example of incorrect identification concerns the vector of groundnut bud necrosis virus, of which *Scirthothrips dorsalis* (Amin

et al., 1981) later turned out to be *T. palmi* (Lakshmi *et al.*, 1995). Also, the vector status of *T. flavus* has not adequately been demonstrated, and, hence, confirmation is needed as this species is easily confused with *T. palmi* as well (Mound, 1996). To avoid incorrect tospovirus vector species determination in the future, an identification key for the reported transmitters is supplied by Mound (1996).

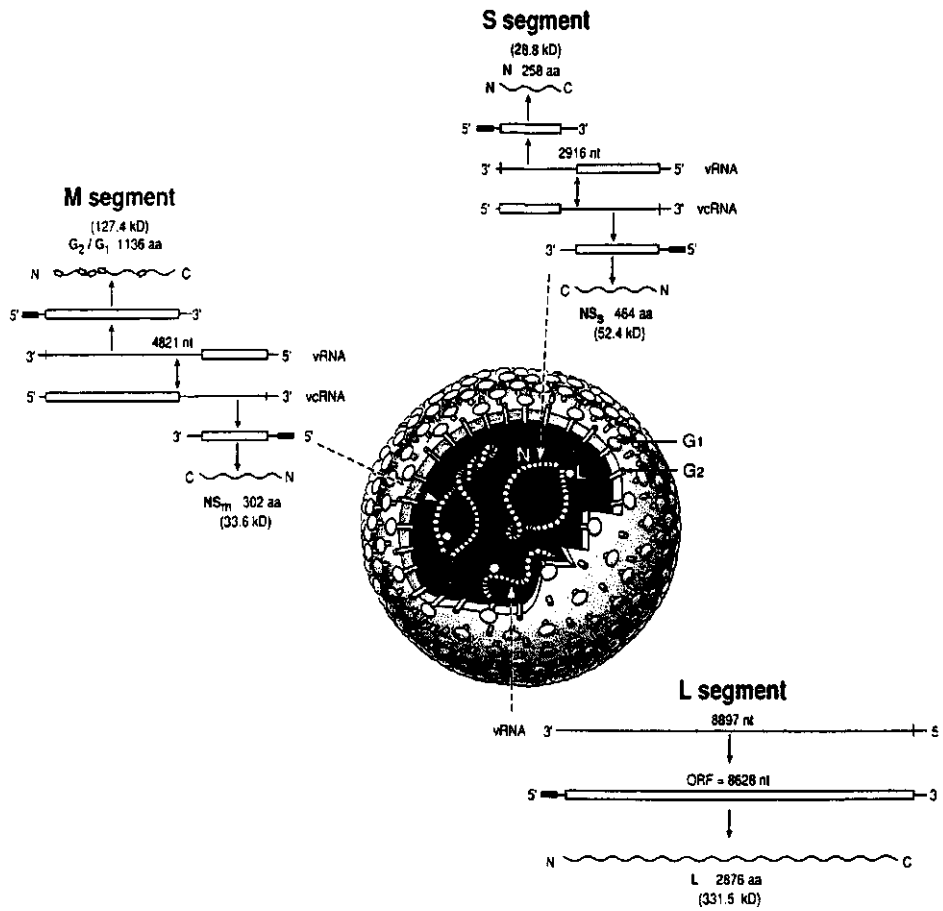

Figure 1. Schematic representation of tomato spotted wilt tospovirus particle. Data are taken from Kormelink *et al.* (1992).

So far, all tospovirus vectors are members of the genera *Frankliniella* and *Thrips*, of the family Thripidae. It is suggested (Mound, 1996) that vector ability is not linked to specific morphological characteristics, but is the result of a rapid evolutionary development between these highly polyphagous thrips species (Anonymous, 1989; Brødsgaard, 1989; Yudin *et al.*, 1986) and tospoviruses.

Frankliniella occidentalis is one of the most efficient vectors of TSWV (Wijkamp *et al.*, 1995). The endemic region of this thrips species was originally California, USA (Moulton, 1931), but nowadays it occurs widespread (Fig. 2) throughout Africa, Asia, Australia, Europe, New Zealand, Polynesian, North, Central and South America (Anonymous, 1993; Dal Bó *et al.*, 1995). The world-wide appearance of *F. occidentalis* is due to its unintentional import on commercial (ornamental) plant material, its increased insecticide resistance, and the large number plant species (at least 244, Anonymous, 1989) on which they are found. In the tropical and subtropical regions, *F. occidentalis* is present in the field, while in regions with a temperate climate its infestations are mainly restricted to greenhouses. In the Netherlands, this thrips species is the most common and most difficult-to-control pest insect in greenhouses, causing feeding damage to greenhouse cultivations of chrysanthemum, cucumber and other crops since 1983 (Mantel and van de Vrie, 1988). Usually, thrips feeding does not immediately diminish crop production, but the resulting scarring (silvery damage) and deformation (growth damage) drastically reduces the aesthetic quality of e.g. chrysanthemum (van Dijken *et al.*, 1994; de Jager *et al.*, 1995), causing less valuable or even unmarketable flowers and plants (Parrella and Jones, 1987).

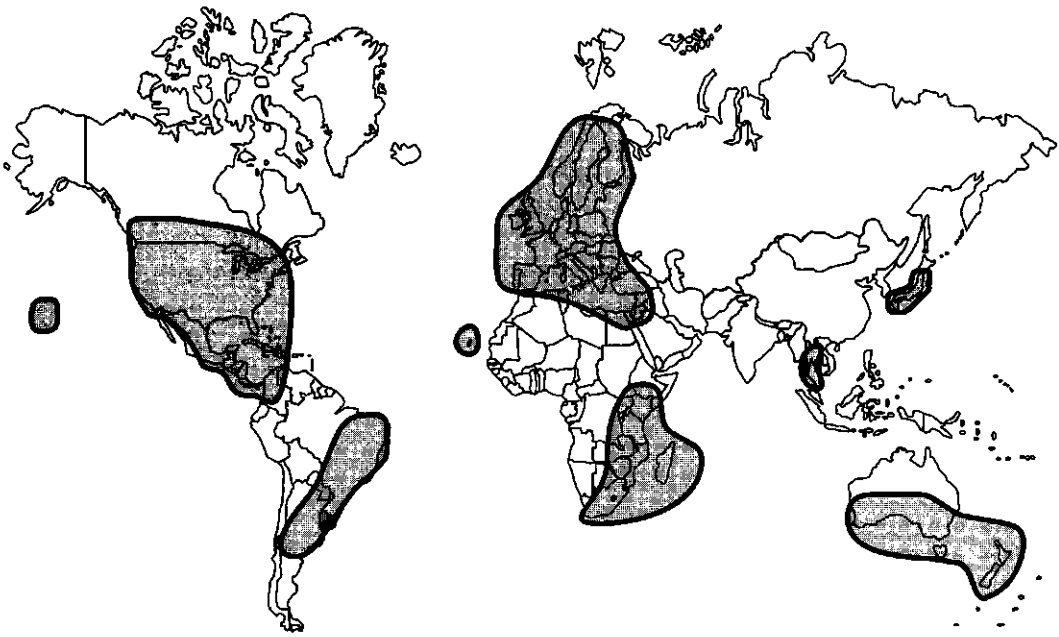

Figure 2. Reported incidence of *Frankliniella occidentalis* (Pergande), after Anonymous (1993) and updated by the present author. To date, likely more and larger areas are infested by *F. occidentalis*, but have not been reported yet.

In addition to inducing direct feeding damage and vectoring tospoviruses, *F. occidentalis* also transmits other pathogens to plants, such as bacteria, fungi and other plant viruses, including ilarviruses, sobemoviruses, and carmoviruses. They carry the pathogen (or virus-infected pollen) on the body surface from one plant to another. Infection is easily established by introduction in thrips injured plant tissues. It should be noted, however, that dissemination of bacteria and fungi (Bournier, 1983) mostly occurs by wind and/or rain (Lewis, 1973).

Besides causing negative effects, *F. occidentalis* can also be beneficial as pollinators or as biological control agents for (other) pest insects and mites by e.g. predating on their eggs (Trichilo and Leigh, 1988). Biological control is defined as the use of a living organism (the beneficial) for the regulation of population size of another (the pest) (Jacobson, 1997). The use of *F. occidentalis* as control agent needs regular inspections in the cropping season to prevent that it develops into a pest itself, when the beneficial effects are no longer balanced by its harmful effects.

Knowledge of the ecology of *F. occidentalis* is essential to understand when or how this thrips species becomes a pest. The life cycle of *F. occidentalis* on bean requires approximately 13 days from egg to adult at 25°C (Wijkamp *et al.*, 1996b), and is dependent on the host plant, temperature and other abiotic factors (Jarošik and Lapchin, 1998). Females insert the eggs into plant tissue using a saw-like ovipositor. Larvae will hatch from the eggs and feed actively on plant tissue as 'feeding-machines'. At the end of the second larval stage, they drop onto the soil or hide in leaf buds, in which they pupate. Adult thrips emerge from the pupae, and feed actively on the plant. *F. occidentalis* populations are bisexual and female-biased. Females are always diploid and males haploid, deriving from fertilised and unfertilised eggs, respectively. *F. occidentalis* females can be visually discriminated from males by their larger size (1.3-1.4 mm and 0.9-1.1 mm from the tip of antennae to the tip of abdomen, respectively (Tommasini and Maini, 1995)), their swollen abdomen due to egg load and melanised cuticle, and the presence of an ovipositor.

Transmission of tospoviruses

Tospovirus transmission is the result of complex interactions between the triad of virus, vector and plant. Only the active feeding stages of thrips are of interest for tospovirus spread, i.e. first (L1) and second instar larvae (L2) and adults (Fig. 3). The TSWV acquisition is restricted to instar larvae (Sakimura, 1963), and after replication and circulation (i.e. propagation) in the thrips (Ullman *et al.*, 1993; Wijkamp *et al.*, 1993), the virus can be transmitted by old L2s and adults (Wijkamp and Peters, 1993). It has been demonstrated that the replication of TSWV has no pathological effects on the thrips (Wijkamp *et al.*, 1996b). Once the thrips is viruliferous it remains so throughout its lifespan.

The adult thrips are known to be the primary dispersal stage, because they are the only thrips stage that can fly, hence, able to infect plants over a large distance.

To date, four types of barriers to TSWV in thrips are identified or suggested, i.e. the midgut (infection and escape) barrier, dissemination barrier, salivary gland barrier and transovarial barrier. A transovarial infection barrier in thrips exists, as it has been demonstrated that TSWV cannot be vertically transmitted (Wijkamp *et al.*, 1996b). Evidence has been obtained that a midgut infection barrier develops when larvae age (Nagata *et al.*, 1999). This barrier is probably regulated by a receptor, a protein of 55 kDa (Bandla *et al.*, 1998). Following the infection of the epithelial cells of the first region of the midgut (Mg1), the virus spreads to the muscle cells of the Mg1 region and the other two midgut regions (Nagata *et al.*,1999). The escape of virus from the midgut is restricted to larvae (Ullman *et al.*, 1992; Ohnishi *et al.*, 1998), suggesting a midgut escape barrier in adults. Little is known about the dissemination or transport of virus from the midgut to the salivary glands, except that a thrips protein of 94 kDa is found that may represent a protein involved in virus circulation through its vector (Kikkert *et al.*, 1998). TSWV virions are found in the salivary glands, salivary ducts and brains of viruliferous larvae and adults (Ullman *et al.*, 1993; Wijkamp *et al.*, 1993), suggesting a salivary gland barrier in nontransmitters. Release of the virus particles into the saliva results in transmission when the thrips feeds.

Successful TSWV transmission only occurs when virus-containing saliva is injected in a viable cell. Whether or not saliva is injected in a cell depends on the feeding behaviour. It is generally accepted that thrips feed in a piercing-sucking manner, and that it includes intervals of probing and non-probing. When not probing, thrips roam, search for new feeding sites or scrape their heads. Because only one canal is present in the mouthparts, food ingestion and saliva egestion can not occur at the same time. Hence, thrips alternate salivation with partial or complete ingestion of cell contents during probing (Chisholm and Lewis, 1984; Harrewijn *et al.*, 1996). Based on the direct damage produced, the probing can be classified into two types: penetration and shallow feeding (Sakimura, 1962). In shallow feeding, damage is limited to epidermal tissues or to a few adjacent mesophyll cells. Here, thrips make a number of punctures, which result in tiny silvery patches, but without obvious scarring of the plant (Chisholm and Lewis, 1984). When thrips make penetration (or intensive) feedings, the content of cells that are located deeper in the mesophyll are emptied, which results in an extreme plasmolysis or complete disappearance of cells. The void space or empty cells are filled with air, causing a silvery, scarred appearance to the affected tissue. It is unknown, whether penetration or shallow feeding favours tospovirus transmission.

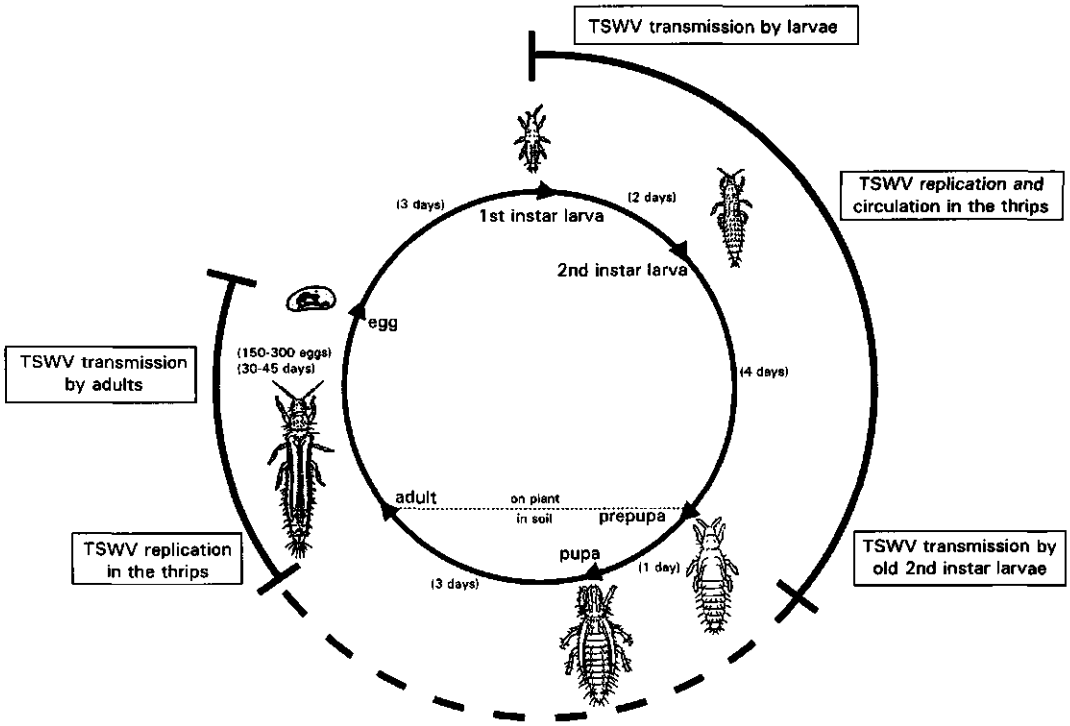

Figure 3. Life cycle of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) at 25°C, with TSWV transmission cycle incorporated.

Tospovirus management strategies

To date, tospovirus control, which is mainly based on thrips management, is difficult and cannot solely rely on a single (chemical) control method, probably as a result of intensive agriculture practices, import of new vectors (e.g. *F. occidentalis*) by increased international trading and –last but not least- the rapid development of pesticide resistance in thrips. The currently available methods to control tospovirus epidemics, which include cultural, physical, chemical and biological measures, are most effective -but never complete- when integrated into pest management programs. These methods are focussed on early identification of tospovirus infected plants and thrips, and on prevention and elimination of primary (i.e. introduction of tospovirus in the crop) and secondary infections (i.e. intercrop spread of tospovirus) in a given production area.

Thrips and tospovirus control requires a different management program for each crop, as the virus introduction and spread is dependent on e.g. the cultivar susceptibility to virus and thrips (Allen *et al.*, 1991; Broadbent and Allen, 1995; Broadbent *et al.*, 1990) and the vector population density (Aramburu *et al.*,1997). The most frequently applied management methods are introduced in the following paragraphs, before the current tospovirus control measures in chrysanthemum are described.

Physical management focuses on virus and thrips identification, prevention of primary infection by screens, and prevention of secondary infection by barriers to intercept thrips that move locally. In most crops, tospovirus infection and spread can be determined by symptom expression on the plants, although latent infections and transient symptoms can hamper identification of infection (Hausbeck *et al.*, 1992). If symptom expression is rather poor, slow or even absent, TSWV infections can readily be determined by collecting several samples of the plants to be inspected using serological assays, such as enzyme-linked immunosorbent assay (ELISA), direct tissue blot and dot blot immunoassays (Hsu and Lawson, 1991). Indicator plants like petunia (Allen and Matteoni, 1991) are tools to identify tospovirus spread and the presence of viruliferous thrips in the cropping area, as symptoms such as black to brown local lesions are found shortly after infection on these plants.

To adequately monitor potential tospovirus vectors -like *F. occidentalis*- in a crop, thrips monitoring is needed. This relies mostly on visual inspection of the crop and on yellow or blue sticky trap cards (Brødsgaard, 1993a and b; Robb and Parrella, 1989), which are particularly useful for detecting thrips at very low densities. Fine mesh screening over greenhouse vents and openings prevent (viruliferous) thrips from entering the production area (Robb and Parrella, 1989), especially when it is 'aluminium-coloured' (Berlinger *et al.*, 1993). The use of barriers like aluminium-surfaced mulch dramatically decreases thrips and virus incidence in tomato, pepper, and tobacco fields with up to 78% (Greenhough and Black, 1990; Kring and Schuster, 1992; Scott *et al.*, 1989), although it is only effective when applied at small, young plants early in the cropping season.

Cultural management is defined as the adjustment of growing practices and environmental conditions to the disadvantage of the pest (Jacobson, 1997), and include nursery hygiene, use of resistant plants, and manipulation of the crop environment. Nursery hygiene is focussed on the prevention of infections using virus- and insect-free stock and planting material, and clean production areas that are free of potential virus and thrips reservoir weeds and (garden) plants and virus infected plants.

For some years, host plant resistance has been implemented in breeding programs as an alternative strategy to diminish tospovirus introduction and subsequent spread. Tospovirus resistance can be based on natural resistance genes or on "pathogen-derived" resistance, in which the plant combats the pathogen with its own, transgenically expressed genes.

So far, natural resistance has been found in a limited number of plant species, i.e. lettuce (Cho *et al.*, 1996), pepper (Black *et al.*, 1991), and tomato (Stevens, 1964; van Zijl *et al.*, 1986). Currently, only one dominant gene of resistance, *Sw-5* (Stevens *et al.*, 1992) has been identified appearing in tomato, making it highly suitable for selective breeding. This gene has been introduced in commercial tomato cultivars in Hawaii and South Africa since 1990 and 1988, respectively. Unfortunately, this resistance has been broken after three years of commercial production in Hawaii (Cho *et al.*, 1996) and five years in South Africa (Thompson and van Zijl, 1996), giving some doubt about its durability. Although genetically engineered (transformed with the N gene of tospovirus[es]), resistant tobacco, tomato, lettuce and chrysanthemum cultivars have been developed (e.g. Daughtrey *et al.*, 1997; de Haan *et al.*, 1996; Pang *et al.*, 1992; Prins *et al.*, 1995; Sherman *et al.*, 1996), they are still not commercially available.

Cultivars with some degree in thrips resistance have already been used in practice to reduce feeding damage (van Dijken *et al.*, 1994; de Jager *et al.*, 1995), reproductive fitness (de Kogel *et al.*, 1997a and c; Mollema *et al.*, 1993; Soria and Mollema, 1995), and size and number of colonising thrips populations. Whether this (partially) resistance will also affect TSWV incidence, is not known yet.

Other cultural management measures are focussed on conventional techniques to manipulate the crop environment, such as crop rotation, use of optimal sowing/planting and harvesting dates (Brown *et al.*, 1998), fertilisation (Brodbeck *et al.*, 1998) and plant density (Brown *et al.*, 1996). These measures can control virus and thrips incidence to some extent in the field, but not to levels that can be obtained with insecticide treatments (Riley *et al.*, 1998).

Control of *F. occidentalis* and tospoviruses requires frequent application of pesticides, and is still frequently used by growers (Stack *et al.*, 1997). However, solely using chemicals does not completely control thrips and TSWV for several reasons. First, *F. occidentalis* rapidly develops resistance to many pesticides (Brødsgaard, 1994b; Robb *et al.*, 1995; Zhao *et al.*, 1995). Second, the inaccessible hide-outs of this species in the plant buds (larvae), medium or soil (pupae) and flowers (larvae and adults) make chemical control of the vector less adequate (Helyer and Brobyn, 1992). Third, thrips can successfully transmit virus in short inoculation access periods (Wijkamp *et al.*, 1996c), before they are killed. Fourth, small numbers of viruliferous thrips can be effective in the spread of these viruses. Fifth, chemical management largely relies upon control of infesting thrips, causing secondary infections, rather than on dispersing thrips, causing primary infections. Studies on several crops have demonstrated that TSWV spread and subsequent losses is mainly caused by dispersing thrips (Camann *et al.*, 1995; Gitaitis *et al.*, 1998; Laviña *et al.*, 1993; Todd *et al.*, 1996). And sixth, pesticide use is declining by consumers' demands, and even

resulting in prohibition of some effective pesticides for commercial practices in the Netherlands, such as dichlorvos.

To date, there are two methods used in thrips management programs: short persistence insecticides, which are specific to the target pest, and systemic insecticides, which have minimal effects on any beneficial organism in the crop. Short-persistence insecticides, such as dichlorvos, kill adult thrips and are mostly introduced soon after planting, in spot treatments of infested plants, and in periods after biological control agents are established. Systemic insecticides are effective against all plant-feeding stages of the thrips and are applied several times during the cropping season (Jacobson, 1997).

Over the years, biological control measures in thrips management programs play a more prominent role in greenhouses but are still of limited value in the field (Parrella and Lewis, 1997). Especially predatory mites and flower bugs (*Orius* species) (Cook *et al.*, 1996; Tavella *et al.*, 1996), and to a lesser degree pathogenic fungi (Parker *et al.*, 1996), are used in integrated management programs. Such integrated programs are needed to achieve the most effective tospovirus control, as biological control alone is not very effective, diminishing only secondary spread but hardly the primary infections (Funderburk *et al.*, 1998).

To date, no specific tospovirus management programs exist for chrysanthemum. It appears difficult to achieve the necessary level of control of *F. occidentalis* as numerous cultivars of different stages are grown in the same production area (Horst and Nelson, 1997). The increased international trading of stock and planting material also results in more frequent introductions of tospoviruses and their vectors in 'clean' greenhouses. The current control recommendations for tospoviruses are to rogue infected plants and to control thrips populations by using clean stock material (Ochoa *et al.*, 1996b), clean production areas (Ochoa *et al.*, 1996a), screens, applications of yellow and blue sticky cards, indicator plants like petunia and the tobacco species *Nicotiana benthamiana* (Daughtrey *et al.*, 1997), the use of thrips resistant cultivars, crop treatments with horticultural oil, insecticidal soap and film-forming products (Allen *et al.*, 1993), and chemical applications (Robb and Parrella, 1989). Insecticides are still repeatedly used, although their use is declining when intensive crop monitoring ensures that chemicals are only applied when really needed (Guldemond and den Belder, 1993; Murphy and Broadbent, 1993). A zero tolerance level for thrips is used by propagators whereas growers typically use counts of 10 thrips per week per 1,000 square feet as a threshold value that signals initiation of spray treatment (Daughtrey *et al.*, 1997).

Control of tospoviruses in chrysanthemum would benefit from the development of tospovirus resistant cultivars and the introduction of beneficial organisms in integrated management programs. Some chrysanthemum cultivars with significant levels of TSWV

resistance have already been developed (Cho *et al.*, 1996; Daughtrey *et al.*, 1997; Sherman *et al.*, 1996), which are now being evaluated by the greenhouse flower industry. Further strategies will be needed and an increased durability of tospovirus control is dependent on knowledge of the intrinsic interactions between tospoviruses, their thrips vectors, and chrysanthemum cultivars.

Outline of the thesis

In view of the problems to adequately control TSWV in chrysanthemum by e.g. the increased resistance of *F. occidentalis* to pesticides and prohibition of effective insecticides, it is necessary to develop alternative control measures in chrysanthemum and to incorporate these in an integrated management program. To realise durability of such a program, an improved understanding of TSWV epidemics in chrysanthemum is needed. Therefore, the role of *F. occidentalis* in TSWV transmission has been intensively studied in this thesis. The research described here was part of a program, supported by the Foundation of Dutch Technology (STW) of the Netherlands Organisation for Scientific Research (NWO), in which also host plant resistance to *F. occidentalis* (de Kogel, 1997; Centre for Plant Breeding and Reproduction Research (CPRO-DLO), Wageningen, the Netherlands) and genetic characterisation of this thrips species and its bacteria (E. J. de Vries, department of Evolution Biology, University of Amsterdam, the Netherlands) were studied with the purpose to gain information on the biology and adaptation ability of *F. occidentalis.*

At the onset of this research, information on tospovirus transmission, with respect to virus acquisition by larvae and transmission by adult thrips, was limited. Experiments were carried out to elucidate this, using the indicator plant petunia in stead of the slow symptom-expressing chrysanthemum. As a first step to an enhanced understanding of tospovirus transmission, the period in which thrips larvae can successfully acquire TSWV and subsequently become transmitters, was determined (Chapter 2). In addition, possible factors influencing this acquisition and transmission ability were investigated, including food ingestion rate, the host plant, tospovirus species and origin of *F. occidentalis* population (Chapter 3). The latter factor was added to elucidate whether *F. occidentalis* populations, collected from fourteen different regions all over the world, would differ in their competency to transmit tospoviruses. Furthermore, studies were made to possibly correlate different thrips feeding behaviour with successful TSWV transmission. The idea that transmission may occur while thrips make (inoculation) punctures, and not by intensive feeding on a single site where cells are left incapable to support virus replication, was studied. Based on differences in size and function, it is assumed that *F. occidentalis* males feed less intensively than females resulting in distinct virus transmission competencies. Hence, TSWV transmission by males and females of fourteen *F. occidentalis* populations

was analysed to determine if differences in tospovirus transmission between sexes exist for all populations, representing a general phenomenon. To elucidate the effects of sex-ratio of a vector population on TSWV spread, the competence of both sexes to transmit virus in time and the adult life-span of both male and female *F. occidentalis* were determined (Chapter 4). Whether or not the distinct feeding behaviour of both sexes would differentially contribute to scar production (result of intensive feeding) was subsequently studied and correlated with TSWV transmission (Chapter 5).

Finally, tospovirus transmission to chrysanthemum cultivars that differed in thrips resistance (based on silvery scar damage rates), was analysed. First, TSWV susceptibility was determined for fifteen cultivars, and an accurate and expeditious assay was developed to overcome the slow symptom expression of chrysanthemum plants (Chapter 6). Using this assay, transmission studies were performed to elucidate whether the use of (partially) resistant cultivars to thrips may add to TSWV control in chrysanthemum (Chapter 7).

Chapter 2

Tomato spotted wilt tospovirus ingestion by first instar larvae of *Frankliniella occidentalis* is a prerequisite for transmission*

Abstract

Tomato spotted wilt tospovirus (TSWV) is, after ingestion by larvae, efficiently transmitted by second instar larvae and adults of the thrips *Frankliniella occidentalis* in a propagative manner. The developmental stage at which thrips larvae acquire an infectious dose, resulting in adults that can transmit the virus, is further defined. TSWV accumulation and transmission occurred after ingestion by first instar larvae. Second instar larvae failed to acquire and retain TSWV upon ingestion and did not develop into transmitters. No correlation was found between the quantity of TSWV ingested by thrips and their ability to acquire TSWV. Instead, first instar larvae gradually lost their acquisition ability with increasing age, suggesting the development of a barrier preventing TSWV acquisition and, consequently, replication needed for transmission. The finding that TSWV is acquired exclusively by first instar larvae of *F. occidentalis*, is of crucial importance for the understanding the epidemiology of this devastating virus.

*This chapter has been published in a slightly modified form as: van de Wetering, F., Goldbach, R. and Peters, D. 1996. Tomato spotted wilt tospovirus ingestion by first instar larvae of *Frankliniella occidentalis* is a prerequisite for transmission. Phytopathology 86: 900-905.

Introduction

Tomato spotted wilt tospovirus (TSWV) ranks among the most devastating plant viruses world-wide (Goldbach and Peters, 1994). The recent resurgence of TSWV, and other tospoviruses (Murphy *et al.*, 1995), is due, in part, to two major factors. Firstly, TSWV has a very broad host range, spanning over 650 plant species including many important vegetable and horticultural crops, as well as numerous weeds, which provides a broad array of ecological niches (Cho *et al.*, 1989; German *et al.*, 1992; Goldbach and Peters, 1994). Secondly, the different thrips species, which transmit TSWV, infest a range of host plants. Currently, seven thrips species have been reported to transmit TSWV (Peters *et al.*, 1996; Webb *et al.*, 1998; Wijkamp *et al.*, 1995), of which *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), the western flower thrips, is a very efficient vector (Wijkamp *et al.*, 1995). This species has expanded its geographical range to almost all (sub) tropical and temperate climate zones of most of the continents (Anonymous, 1993; Brødsgaard, 1989; Dal Bó *et al.*, 1995). Over the past few years the control of *F. occidentalis* became more difficult due in part to its resistance to many chemical insecticides. In addition, the incidence of TSWV cannot be controlled by insecticides applied to incoming thrips (e.g. Laviña *et al.*, 1993; Todd *et al.*, 1996). Host plant resistance and biological control could possibly provide alternatives to control thrips and satisfy consumers' demands for a decrease in the use of pesticides (Bakker and Sabelis, 1989; de Ponti, 1982; Mollema *et al.*, 1990). To develop sustainable management strategies for control of thrips and TSWV epidemics, a more detailed understanding of the TSWV-infection cycle and virus-vector interactions are essential (Cho *et al.*, 1989).

It is generally accepted that only thrips larvae acquire the TSWV (Sakimura, 1962). After ingestion and subsequent virus multiplication (Ullman *et al.*, 1993; Wijkamp *et al.*, 1993), TSWV can be transmitted by second instar larvae (L2s) as well as adults (Wijkamp and Peters, 1993). Once the vector becomes viruliferous, it remains so throughout its lifespan, but the rate at which each individual transmits may vary considerably (Sakimura, 1962; Wijkamp *et al.*, 1996b).

In this report the developmental stages at which thrips larvae acquire and transmit TSWV were analysed and defined. The results show that only first instar larvae of *F. occidentalis* can acquire TSWV after ingestion and that these thrips become transmitters later in their development. Possible explanations for the exclusive capacity of first instar larvae to acquire TSWV are discussed.

Materials and methods

Thrips

The *F. occidentalis* used in our studies were obtained from infested bean plants in a greenhouse in the Netherlands and were reared as virus-free colonies on bean pods (*Phaseolus vulgaris* L.) at 27 ± 0.5°C and a 16 h photoperiod each day.

TSWV isolate and plant material

In all experiments, the Brazilian TSWV isolate BR-01 (de Ávila *et al.*, 1990) was used. This isolate was maintained by thrips inoculation on *Datura stramonium* L. plants. Plants used in acquisition experiments were mechanically inoculated on the first two leaves with extracts from thrips-inoculated plants. These plants were kept in a greenhouse at approximately 22°C (16/8 h of light/dark).

Preparation of antiserum and conjugate for enzyme-linked immunosorbent assay (ELISA)

Polyclonal antiserum raised against the nucleocapsid protein (N protein) of TSWV isolate BR-01 was used in ELISA (de Ávila *et al.*, 1992). The immunoglobulin G (IgG) fraction was partially purified by ammonium sulphate precipitation (Clark and Adams, 1977). IgG was conjugated at a concentration of 1 mg/ml with 2,000 units of alkaline phosphatase (Grade I, Boehringer, GmbH, Mannheim, Germany) in phosphate-buffered saline (PBS; 0.14 M NaCl, 1 mM KH_2PO_4, 8 mM Na_2HPO_4, 2.5 mM KCl), pH 7.4 (Avrameas, 1969). The IgG and conjugate were stored with 0.05% sodium azide at 4°C.

TSWV-N protein detection by ELISA

The presence of TSWV-N protein in individual thrips was detected using a cocktail ELISA with enzyme amplification (Resende *et al.*, 1991; van den Heuvel and Peters, 1989). Wells of Nunc-Immunoplate Maxisorp F96 plates (Nunc, Roskilde, Denmark) were coated with 150 µl of 0.5 µg of IgG per ml in coating buffer (0.05 M sodium carbonate, pH 9.6). After incubation overnight at 4 C, the plates were rinsed three times with PBS-Tween (PBS with 0.05% Tween-20). Individual thrips were placed in Eppendorf tubes and ground with a micropestle in 100 µl of sample buffer (2% polyvinylpyrrolidone [M_r about 44,000] and 0.2% ovalbumin in PBS-Tween). The samples were added to the wells and mixed with 25 µl of 1 µg of TSWV anti-N protein conjugate per ml in sample buffer. Virus-free thrips and a dilution series of a nucleocapsid preparation purified from TSWV infected *Nicotiana rustica* plants were used as the control and standard, respectively. The cocktail was incubated overnight at 4°C and, afterward, was rinsed three times with PBS-Tween.

The enzyme amplification reaction was performed as described by van den Heuvel and Peters (1989). Initially, 100 µl of 0.2 mM NADP monosodium salt in 0.05 M diethanolamine buffer, pH 9.5, was added to each well. After incubation at room

temperature for 45 min, 140 µl of the amplification mixture was added per well. The stock amplification mixture consisted of 700 units of alcohol dehydrogenase and 100 µl 1 mM *p*-iodonitrotetrazolium violet in 1 ml of 0.025 M phosphate buffer, pH 7.0. The reaction was allowed to proceed at room temperature. Absorbance values were determined with an EL312 ELISA-reader (Bio-teck Instruments, Greiner BV, Alphen aan de Rijn, the Netherlands) at 490 nm. The values were corrected for blank values read for wells that contained only sample buffer in the sample incubation step. The threshold value was calculated as the average of at least six virus-free thrips plus 3 times s.d. All the readings above the threshold value were considered positive. The amount of TSWV-N protein found in each thrips was calculated by the values of the dilution series of nucleocapsid preparations.

Handling thrips in transmission experiments

Systemically infected leaves of *D. stramonium* plants that showed equally high TSWV-N protein titers in a dilution series, with double antibody sandwich (DAS)-ELISA (Clark and Adams, 1977; Resende *et al.*, 1991), were used as sources for acquisition. The infected leaves were cut in pieces and placed randomly in Tashiro cages (Tashiro, 1967) in which *F. occidentalis* larvae were confined. Separate groups of instar larvae were caged on virus-free plant material as control treatments. After the acquisition access period (AAP), the thrips were transferred to virus-free *D. stramonium* plant material, and allowed to complete their development until adult emergence. All the experiments were carried out at 25 ± 0.5°C. Adult stage thrips were individually tested for TSWV transmission on leaf disks (13 mm in diameter) of *Petunia x hybrida* cv. 'Polo Blauw' as previously described (Wijkamp and Peters, 1993), for three successive inoculation access periods (IAP) of 48 h. After each IAP, the leaf disks were floated on water for 2 days at 27 ± 0.5°C in 24 well plates (Costar Europe Ltd., Badhoevedorp, the Netherlands) for symptom development. Transmission efficiency was calculated as the percentage of leaf disks that developed local lesions (van de Wetering *et al.*, 1996).

TSWV transmission experiments

Newly hatched and 1-, 2-, 3-, 4-, and 5-day-old thrips larvae were placed on TSWV-infected material for 24 h, using in total 58, 71, 51, 40, 51 and 50 insects, respectively, in two replications. Before and after the AAPs the thrips were kept on virus-free *D. stramonium* plant material. Transmission efficiency was expressed as the number of adults that transmitted TSWV, using the petunia local lesion assay. Adult thrips were subsequently stored at −70°C, prior to ELISA. Samples of thrips that fed on noninfected plant material were used as controls. The ELISA values for thrips were analysed using

Duncan's multiple range test with a STATGRAPHICS 6.0 PLUS computer program (Rijpkema, 1993; Schulman, 1992).

In another experiment, 0-, 12-, 20-, 28-, 36-, 44-, 52-, 56-h-old larvae were given a 24-h AAP with 30 insects per treatment per replication. Prior to AAP, the ratio of first instar larvae (L1s) and L2s was determined by counting the moulting skins found in each treatment. Transmission efficiency was determined for adults. This experiment was repeated three times. Thrips grown on virus-free material were used as controls.

Ingestion and transmission by L1s and L2s

Newly hatched, 0-h-old L1s ($n=84$), 60-h-old L2s ($n=102$) and 40-h-old larvae ($n=138$) were placed for 4 h on infected material. Before and after the AAP, the larval stage of the 40-h-old larvae was determined based on the moulting skins and gut colour. The gut has a green colour during feeding on *D. stramonium* and turns transparent during the moulting stage. Using this qualitative technique together and the moulting skins found, the larvae were divided into four categories, i.e., L1s ($n=26$), L2s ($n=21$), larvae in the moulting stage ($n=45$), and a mixture of L1s and L2s ($n=46$). After the AAP of 4 h, larvae from each group were collected randomly, frozen at −70°C, and later assayed by ELISA. Transmission efficiency was determined for adults by the petunia local lesion assay.

TSWV accumulation in maturing thrips

TSWV accumulation was analysed in maturing thrips after they ingested virus as newly hatched L1s and 2.5 day-old L2s. The thrips were given a 4-h AAP on TSWV-infected *D. stramonium* plant material. Respective groups of 25 individuals were collected randomly immediately after AAP every day in the larval, the pupal and the adult stages. These thrips were frozen immediately at −70°C and later tested individually for the presence of N protein. Thrips that were confined for 4 h on noninfected *D. stramonium* plant material, were used as controls.

Determination of the amount of TSWV ingested

Larvae ranging in the age from 0 to 5 days old were given an 8-h AAP on TSWV-infected *D. stramonium* plant material. Before the AAP, the thrips were kept on virus-free *D. stramonium* plant material. Immediately after the AAP, the larvae (approximately 25 per treatment) of each age group were collected and stored at −70°C, until tested by ELISA. Samples of thrips confined for 8 h on virus-free *D. stramonium* plant material were used as controls.

Results

Relation between ingestion of TSWV by larvae and transmission by adults

We allowed different developmental stages of *F. occidentalis* larvae, ranging between 0 to 5 days old, to ingest TSWV to compare the ability of the adult-stage thrips to transmit TSWV. Our data showed that TSWV acquisition by 0- to 2-day-old larvae resulted in adults that were capable of transmitting TSWV, whereas when 3- to 5-day-old larvae fed on TSWV-infected plant material the adults failed to transmit TSWV (Fig. 1). The highest adult transmission rate was obtained when newly hatched larvae had ingested TSWV. Transmission efficiency decreased sharply with increase in the age at which L1s fed on infected plant material. Also, when thrips were tested for TSWV-N protein by ELISA, the number of ELISA-positive adults decreased with increasing age at which TSWV was ingested by L1s, and paralleled the decrease in adult transmission efficiency.

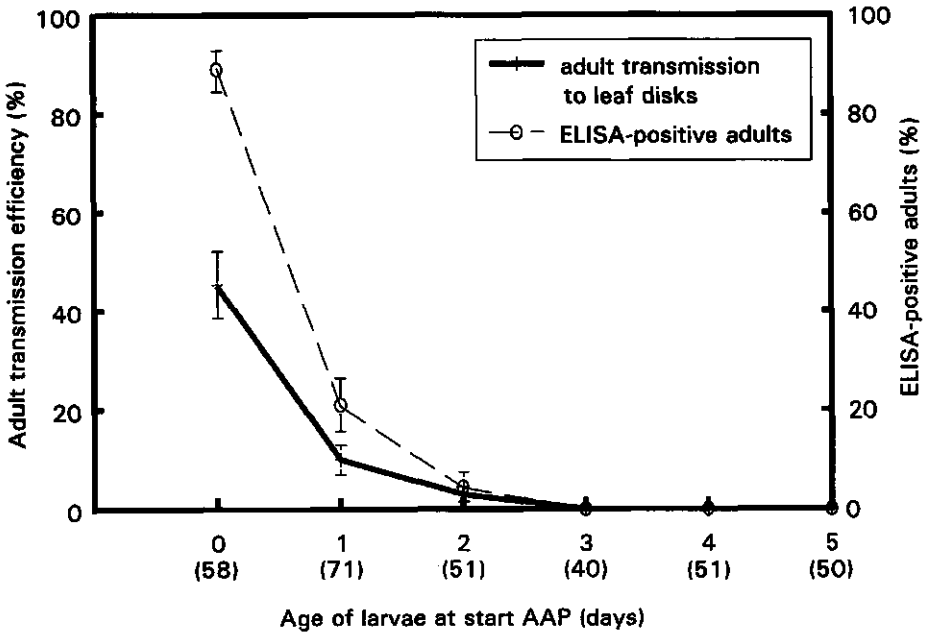

Figure 1. Tomato spotted wilt virus (TSWV) transmission efficiency (± s.e.) and enzyme-linked immunosorbent assay (ELISA)-positive adult thrips after a 24-h acquisition access period (AAP) on TSWV-infected *Datura stramonium* plant material by 0- to 5-day-old larvae. Values below x-axis represent the number of thrips tested and are the sum of two replications.

Ninety percent of the adults that fed as newly hatched larvae were ELISA-positive, and 48% transmitted TSWV. No adult thrips that ingested TSWV as 3- to 5-day-old larvae were ELISA-positive. The results also showed that ELISA values for adult thrips (which were TSWV transmitters versus nontransmitters) were compared, all thrips transmitters showed a significantly higher ELISA value than the nontransmitters ($P<0.05$), hence a certain threshold amount of TSWV might need to be present in order for successful transmission (Table 1). Furthermore, thrips that were nontransmitters could be divided into adults that were ELISA-positive and -negative. ELISA-positive thrips were found only when TSWV was fed to 0- to 2-day-old larvae.

Table 1. Average enzyme-linked immunosorbent assay (ELISA) values (± s.e.), using antiserum against tomato spotted wilt virus-nucleocapsid protein (TSWV-N protein), for adult thrips after TSWV ingestion by larvae of different ages.

Age of acquisition (days)	ELISA values[1]		
	Transmitters	Non-transmitters	
		ELISA-positive	ELISA-negative
0	0.785 ± 0.024^{a} (22)	0.390 ± 0.014^{b} (31)	0.001 ± 0.005^{d} (5)
1	0.723 ± 0.111^{a} (2)	0.219 ± 0.103^{bc} (9)	0.002 ± 0.001^{d} (60)
2	0.757^{a} (5)	0.046^{cd} (1)	0.001 ± 0.002^{d} (45)
3	--	--	0.004 ± 0.002^{d} (40)
4	--	--	0.002 ± 0.002^{d} (51)
5	--	--	0.003 ± 0.001^{d} (50)

[1] Transmitter: thrips that transmitted TSWV to petunia. All were ELISA-positive. Nontransmitter: non-transmitting thrips were divided into two categories based on their readings in ELISA. The threshold value was calculated as the average of virus-free thrips + 3 times s.d. All readings above this threshold, ranging from 0.009 to 0.025, were considered positive; readings below this value were considered negative. Values followed by the same letter are not significantly different according to Duncan's multiple range test ($P<0.05$). The number of insects tested per combination is indicated in parentheses and is the sum of two replications.

In the next type of experiments, we studied acquisition of TSWV, using groups of larvae, ranging in age from 0 to 56 h, to define more precisely the moment at which the larvae lost their ability to acquire TSWV. The L1/L2 ratio of larvae, age of larvae when fed on TSWV-infected plant material, and ability to transmit TSWV in the adult stage were determined. The highest transmission efficiency (42%), again, was observed for adults that fed as newly hatched larvae on TSWV-infected plant material. Larvae by the age of 56 h (L2s) could not acquire TSWV, because when they were adults they did not transmit the virus. These results, again, show that the ability to acquire TSWV decreased with age of L1s, and that the potential to acquire TSWV was significantly reduced for L2s. The data, however, cannot exclude the possibility that some early L2s acquired TSWV, because some were present in the 36- and 44-h-old populations analysed (Fig. 2).

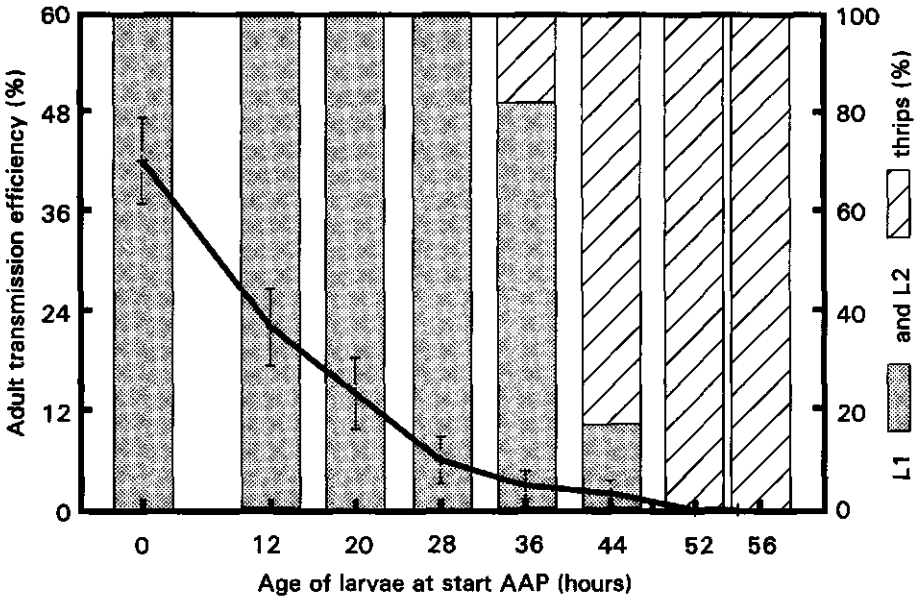

Figure 2. Relationship between L1 (first instar larvae)/L2 (second instar larvae) ratio of thrips at acquisition and the subsequent transmission efficiency of the resulting adults. Larvae between 0- to 56-h-old were given a 24-h acquisition access period (AAP) on tomato spotted wilt virus (TSWV)-infected *Datura stramonium* plant material. Averages ± s.e. are presented for three replications, with 30 insects per treatment per replication.

TSWV acquisition by thrips larvae

Apparently, only L1s are able to acquire TSWV. To more definitively determine whether early L2s could acquire and retain TSWV, 0-h-old larvae (L1s), 60-h-old larvae (L2s) and 40-h-old larvae (L1s and L2s) were allowed a 4-h AAP on TSWV-infected plant material. TSWV ingestion by individual larvae, using 26 L1s, 51 L1 or L2s and 25 L2s, was monitored by ELISA. The adult transmission efficiency of the remaining thrips was determined on petunia leaf disks (Table 2). The developmental stage of 40 h-old larvae was determined by counting the moulting skins and using gut colour before and after AAP as an indicator for feeding. Four groups were distinguished by assessing the gut colour. One group ($n = 26$) consisted of larvae that had a green gut before and a transparent gut after the AAP. Because they may have moulted during AAP, they were considered L1s before the AAP. A second group ($n = 45$) consisted of larvae in which the gut was transparent before and after the AAP. They were evidently in the L1-L2 moulting stage. The third group ($n = 21$) contained L2s that had a transparent gut before and a green gut after AAP. Larvae of the fourth group ($n = 46$) had a green gut before and after the AAP, and represented a mixture of L1s and L2s (Table 2).

Table 2. Tomato spotted wilt virus (TSWV)-ingestion by 0-, 40-, and 60-h-old thrips larvae and subsequent transmission by the corresponding adults.

	Larvae gut colour Before and after AAP[1]		Larvae after AAP (ELISA)[2]		
Age of larvae (h)	Before	After	Positive larvae[3]/ total tested	Amount of N protein ingested (ng)[4]	Transmitting adults[5]/ Total tested
0	Transparent	Green[6]	23/26	3.6 ±0.8	31/58
40	Green	Transparent[6]	8/13	0.6 ±0.1	1/13
	Transparent	Transparent[7]	0/13	0.0	0/32
	Transparent	Green[8]	11/11	1.3 ±0.4	0/10
	Green	Green[9]	11/14	5.4 ±0.9	0/32
60	Green	Green[8]	23/25	10.8 ±4.7	0/77

[1] Gut colour was used, in combination with counting moulting skins, to identify larval stage that had been given a 4-h acquisition access period (AAP); [2] Enzyme-linked immunosorbent assay; [3] Larvae that contained nucleocapsid protein; [4] Average ± s.e.; [5] TSWV transmission to petunia leaf disks; [6] L1 stage (first instar); [7] Moulting stage of L1 to L2 (second instar); [8] L2 stage; [9] Mixture of L1s and L2s.

Newly hatched larvae ingested, on average, 3.6 ng of TSWV-N protein, as measured immediately after a 4-h-AAP, and 53% of the thrips in this population transmitted TSWV after becoming adults. Second instar larvae of 60 h old contained, on average, 10.8 ng of N protein immediately after the AAP but failed to transmit TSWV in the adult stage. At the age of 40 h, the old L1s after AAP contained, on average, less TSWV-N protein than newly hatched larvae, and only one adult was able to transmit TSWV. After AAP, no N protein was detected in larvae which were in process of moulting. Adults emerging from these larvae failed to transmit TSWV. The thrips of the third group, consisting of early L2s, contained, on average, lower amounts of N protein (1.3 ng) than 60 h-old larvae (10.8 ng), but more than old L1s (0.6 ng) (Table 2). Although the early L2s did ingest virus, the adults that developed from these larvae did not become transmitters. An average of 5.4 ng N protein was detected in the larvae from the fourth group. It is possible that they might represent a mixture of L1s and L2s. The level of TSWV-N protein was higher in these larvae than in 40 h-old first instar larvae. Therefore, it seems likely that this group of 40-h-old larvae consisted mainly of L2s. The results obtained in these analyses show that only L1s acquire TSWV.

TSWV accumulation in maturing thrips

TSWV accumulation was analysed in thrips after they ingested TSWV as newly hatched (L1s) and 2.5-day-old larvae (L2s) to explain the exclusive capacity of first instar larvae to acquire TSWV. The L1 and L2 larvae were given a 4-h AAP on TSWV-infected plant material. Samples of 25 individuals were collected randomly at intervals during their development into adults and analysed individually in ELISA for the presence of TSWV-N protein (Fig. 3). On average, 3.6 ng of N protein was detected in L1s, when sampled directly after the AAP. Four-day-old thrips contained an average of 13.1 ng N protein. A decrease in the level of N protein was detected in the pupal stage (1.5 ng), and, again, an increase of N protein was detected in the adults (16.3 ng). The L2s, however, ingested more virus (10.8 ng of N protein on average), but no increase in amount of this protein could be detected during their development (Fig. 3).

Influence of amount of virus ingested on ability to acquire TSWV

The loss of acquisition capacity with age may be explained by the relative amount of TSWV- N protein ingested by larvae during development. To study TSWV ingestion, larvae ranging in age from 0 to 5 days were placed on TSWV-infected plant material for 8 h. Immediately after AAP, the thrips were frozen at -70°C, and later tested for their N protein content (Fig. 4a). The results show that the amount of N protein after AAP increased with the age of the larvae, and, hence, reflects an increasing consumption rate with age. The amount of N protein ingested by larvae, therefore, does not appear to determine or

significantly influence the resulting potential of adults to become transmitters. Finally, at all ages, some individuals showed no evidence of having ingested TSWV, as determined by ELISA done immediately after AAP (Fig. 4b).

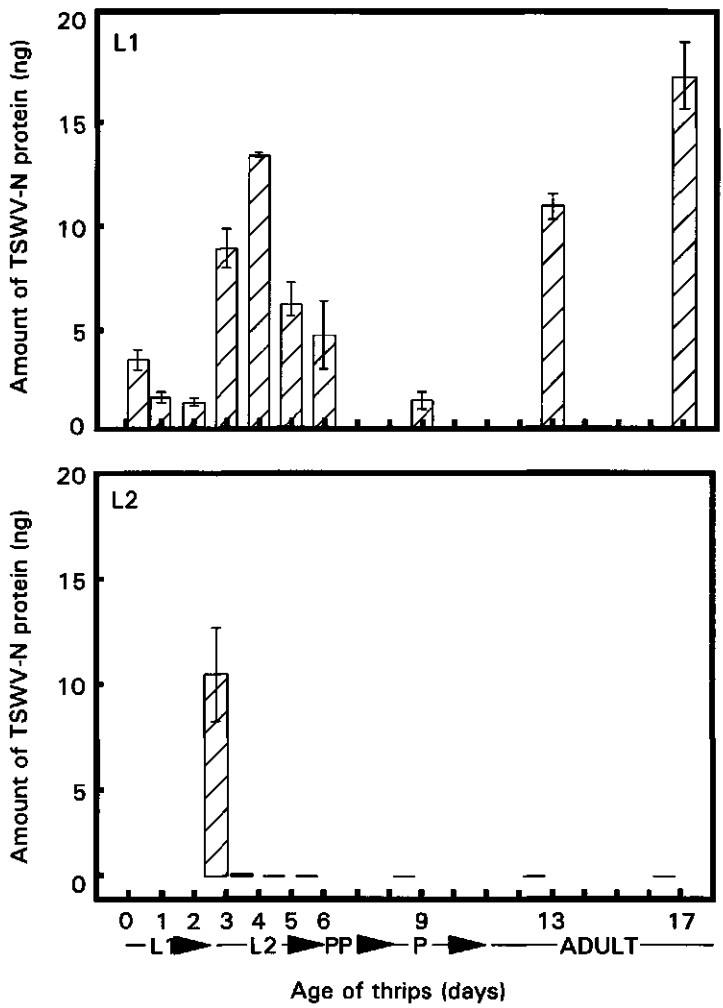

Figure 3. Detection of tomato spotted wilt virus-nucleocapsid protein (TSWV-N protein) in maturing thrips ($n = 25$ per treatment; newly hatched larvae [L1] and 2.5-day-old larvae [L2]) after a 4-h acquisition access period (AAP) on TSWV-infected *Datura stramonium* plant material. Amounts of TSWV-N protein higher than the threshold value, ranging from 0.005 to 0.050 ng, were considered positive. The average amount of N protein, determined by enzyme-linked immunosorbent assay (ELISA) values ± s.e., is shown.

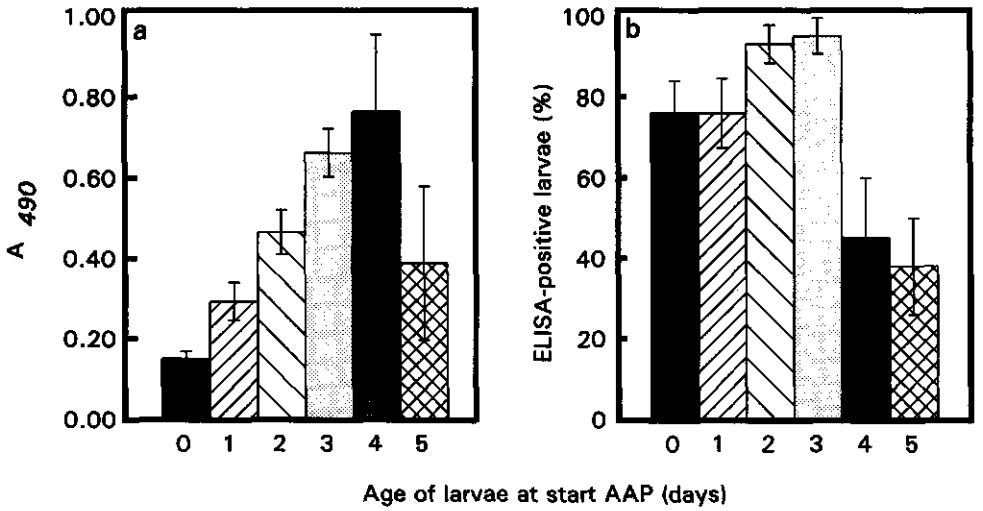

Figure 4. The average enzyme-linked immunosorbent assay (ELISA) values ± s.e., using antiserum against tomato spotted wilt virus-nucleocapsid protein (TSWV-N protein), of individual larvae (a), and the percentage of larvae which were ELISA-positive for TSWV (b). ELISA values higher than the threshold value, ranging from 0.006 to 0.011, were considered positive. Newly hatched to 5-day-old larvae were given an 8-h acquisition access period (AAP) to TSWV-infected *Datura stramonium* plant material ($n = 25$ per treatment).

Discussion

The results presented in this paper clearly show that TSWV ingestion by L1s of *Frankliniella occidentalis* is a prerequisite for transmission by later stages (Figs. 1 and 2, Tables 1 and 2). The percentage of adults that transmitted TSWV decreased sharply when older L1s were used in TSWV acquisition experiments (Fig. 2). The exclusive capacity of L1s to acquire TSWV was explained by the ability to accumulate TSWV in later stages after ingestion, whereas second instar larvae failed to do so (Fig. 3). No correlation was found between the amount of TSWV ingested and the ability of thrips to acquire TSWV (Figs. 4a and b).

A novel technique, in combination with counting moulting skins, was used to discriminate between old L1s and early L2s, because they are difficult to differentiate by any morphological characteristic (Brødsgaard, 1994a; Malchau, 1990; van Rijn *et al.*, 1995). Previously described methods (Malchau, 1990; van Rijn, 1995) were based on counting moulting skins (Fig. 2), which alone is not a highly accurate technique, because

larvae also feed on moulting skins. The new technique presented here is based on qualitatively scoring gut colour. During the moulting stages, thrips have a transparent gut, whereas it is green during feeding. The combination of assessing gut colour and counting moulting skins can very accurately distinguish between L1s and L2s (Table 2).

The fact that only L1s could acquire TSWV suggests that a barrier exists in second instar larvae and at later stages that prevents acquisition and subsequent TSWV replication needed for transmission. To date, four types of barriers to propagative plant viruses in insects are known, i.e. the midgut barrier, dissemination barrier, salivary gland escape barrier, and transovarial and venereal transmission barriers (Ammar, 1994; German *et al.*, 1992). The failure to infect the midgut in second instar larvae, and even in later stages, might be explained by an inactivation of TSWV by digestive or proteolytic enzymes in the gut lumen, encapsulation of the virus in the peritrophic membrane (Gildow and Gray, 1993), or blocking of possible TSWV-receptors (Bandla *et al.*, 1995; Laviña *et al.*, 1993; Ludwig *et al.*, 1989; Ludwig *et al.*, 1991).

The differences between adults and larvae in the ability to acquire TSWV have already been investigated histologically by Ullman *et al.* (1992), who suggested that a midgut barrier, preventing TSWV dissemination into the hemocoel (Ullman *et al.*, 1995), exists in the adults but not in larvae. In the future, similar experiments need to be executed to determine whether this barrier is present in L2s. Further research also might provide insight into a possible third barrier: the salivary gland escape barrier. The existence of transovarial and venereal barriers has been demonstrated already, because female thrips cannot transmit TSWV vertically (Wijkamp *et al.*, 1996b).

Larval instars of aphids, leafhoppers, and planthoppers acquire circulative and propagative viruses more efficiently than adults (Ammar, 1994; Sylvester, 1980). However, a principal difference between larvae and adults and even between larval stages in the ability to acquire virus has been described only for Fiji disease reovirus (FDV) which is transmitted by the planthoppers *Perkinsiella saccharicida*, *P. vitiensis*, and *P. vastatrix*. This virus can be acquired only by the L1s, L2s and L3s, and not by L4s, L5s and adults. As with TSWV, the ability to acquire an infectious dose of FDV also decreases with increasing larval age (Anonymous, 1979; Daniels *et al.*, 1969; Egan *et al.*, 1989; Francki *et al.*, 1986; Mungomery and Bell, 1939; Murphy *et al.*, 1995). Ammar (1994) suggested the occurrence of a midgut (infection or escape) barrier or other dissemination barriers for FDV in the later instar and adult stages.

The finding that only L1s of *F. occidentalis* can acquire and retain TSWV and that only these individuals develop into transmitters is crucial for the understanding TSWV epidemiology. Our data allow us to describe the TSWV-thrips transmission cycle more accurately. TSWV must be ingested by L1s, and after a latent period and virus replication, TSWV subsequently can be transmitted by old L2s and adults (Ullman *et al.*, 1993;

Wijkamp and Peters, 1993; Wijkamp *et al.*, 1993). In defining the TSWV-thrips transmission relationship more accurately, our findings are relevant for designing optimal TSWV control measures. Control of TSWV, at the moment, is focussed on the transmitters, whereas control possibly could be more effective if aimed at the beginning of the transmission cycle, i.e., targeting the L1s.

Acknowledgements

This research was supported by a fellowship ABI22.2704 of the Foundation of Dutch Technology (STW) of the Netherlands Organisation for Scientific Research (NWO). We thank S&G Seeds, Enkhuizen, the Netherlands, for supplying us with *Petunia x hybrida* cv. 'Polo Blauw' seeds. We thank P. Krell for critical reading of the manuscript.

Chapter 3

Variation in tospovirus transmission between *Frankliniella occidentalis* populations*

Abstract

Fourteen western flower thrips (*Frankliniella occidentalis*) populations, originating from countries in Asia, Europe, North America and from New Zealand, were analysed for their efficiency to transmit tomato spotted wilt tospovirus (TSWV). All populations acquired and subsequently transmitted the virus, but a marked variation was found in transmission efficiency. This efficiency, expressed as the percentage of transmitting adults of both sexes, varied from 18% for a population from the USA (US2) to 75% for a population from Israel (IS2). These differences between populations were not affected by the amount of virus ingested or by the host plant used. However, the age of larvae at which the virus was acquired and the tospovirus species studied directly influenced the virus transmission efficiencies. It could be confirmed (see also Chapter 2) that only first instar larvae of the NL3 population were able to acquire TSWV, whereas second instar larvae failed to do so. However, both instar larvae of this population were able to acquire another tospovirus, impatiens necrotic spot virus (INSV). The IS2 and US2 populations could also acquire TSWV as well as INSV in both their first and second larval stages, although the ability to acquire virus decreased with increasing larval age. Hence, it is likely that, in general, both instar larvae of *F. occidentalis* have the capacity to acquire tospoviruses.

*This chapter has been submitted for publication to Bulletin of Entomological Research as: van de Wetering, F., M. van der Hoek, R. Goldbach, C. Mollema, and D. Peters. Distinct differences in tospovirus transmission between *Frankliniella occidentalis* populations.

Introduction

Tospoviruses cause serious diseases in crops cultivated in the open field and in greenhouses throughout tropical, subtropical and temperate climate zones (German *et al.*, 1992; Peters *et al.*, 1996). Of all tospoviruses, tomato spotted wilt virus (TSWV), type species of the genus, is most predominant. Due to its large range, rapid expansion and difficult control of its vectors, this virus ranks among the top ten of economically most important plant viruses, causing crop losses world-wide for more than 1 billion US dollars a year (Goldbach and Peters, 1994).

Tospoviruses are transmitted by thrips (Thysanoptera: Thripidae), minute insects of approximately 1 mm long. In the 1930s and 40s, the major vector of TSWV was reported to be the onion thrips, *Thrips tabaci* (Lindeman) (Pittman, 1927). Diseases caused by TSWV faded away almost completely in western Europe in the 50s probably due to effective and intensive chemical control of this thrips species. A renewed incidence of TSWV in the 1980s has been attributed to unintentional import, to increased pesticide resistance (Brødsgaard, 1994b; Robb *et al.*, 1995; Zhao *et al.*, 1995) and to dispersal of a new effective vector, the western flower thrips, *Frankliniella occidentalis* (Pergande). This thrips species was originally known as a local pest in the western part of the USA (Moulton, 1931), but to date its occurrence has been reported in throughout North and Central America, Africa, Australia, Europe, the Middle East, south-east Asia and New Zealand (Anonymous, 1993; Brødsgaard, 1989; Dal Bó *et al.*, 1995). The expansion of *F. occidentalis* was associated with the occurrence of another, but distinct tospovirus, impatiens necrotic spot virus (INSV) in the United States (Law and Moyer, 1990) and, soon later, in Europe (de Ávila *et al.*, 1992).

To date, already more than eight distinct tospovirus species have been recognised (Goldbach and Kuo, 1996), which are transmitted by at least eight thrips species (Webb *et al.*, 1998; Wijkamp *et al.*, 1995). Of these vectors, *F. occidentalis* is one of the most efficient transmitters of TSWV and INSV (Wijkamp *et al.*, 1995).

Unfortunately, tospovirus spread cannot be controlled by the current management strategies (e.g. Daughtrey *et al.*, 1997; Todd *et al.*, 1996). The development of alternative and durable strategies to control tospoviruses and their vectors is dependent on an improved understanding of tospovirus epidemics, and hence of tospovirus-vector interactions. Although many transmission parameters have been elucidated, including the propagative mode of transmission (Ullman *et al.*, 1993; Wijkamp *et al.*, 1993) after acquisition by young larvae (Chapter 2), information on possible differences in virus transmission competencies by thrips populations originally from different biological niches, was lacking. In view of the global spread of *F. occidentalis*, its ability to infest many plant species and its quick adaptation ability to different environmental conditions, variation may exist between the various *F. occidentalis* populations in their ability to acquire and transmit

tospoviruses. In this report, tospovirus transmission by fourteen *F. occidentalis* populations, which originated from different regions, is presented. A number of these populations were selected to investigate whether factors like the larval age when ingesting the virus, the amount of virus ingested, tospovirus species and plant species affect the virus transmission competencies.

Materials and methods

Origins and maintenance of thrips colonies

Adults of the different *F. occidentalis* populations were obtained from Centre for Plant Breeding and Reproduction Research (CPRO-DLO) Wageningen and our laboratory (NL3). The populations were collected from various crops and locations throughout the world (Table 1).

Table 1. Geographical areas and originated hosts of *Frankliniella occidentalis* populations analysed.

Population designation	Country	Crop	Collector
DK	Denmark	Bean	Brødsgaard
FR	France	Bean	Leclant
IS1	Israel	Strawberry	Klein
IS2	Israel	Mango	Klein
IT	Italy	bean	Tommasini
JA	Japan	chrysanthemum	Murai
NL1	the Netherlands	cucumber	Mollema
NL2	the Netherlands	chrysanthemum	Van Dijken
NL3	the Netherlands	bean	van de Wetering/ Wijkamp
NL4	the Netherlands	tomato	Ramakers
NL5	the Netherlands	rose	Fransen
NZ	New Zealand	egg plant	Martin
US1	United States	gloxinia	Baker
US2	United States	chrysanthemum	Shearin

These populations were maintained for at least two-and-a-half years as virus free colonies on bean pods (*Phaseolus vulgaris* L. cv. Prelude) at 27 ± 0.5°C (16/8 h of light/dark), before this study was made (de Kogel *et al.*, 1997c). To avoid contamination with thrips from other origins, the pods were washed in water, incubated at 27 ± 0.5°C for four days, and washed again to remove hatched larvae before the pods were used in the rearings. The sex ratio of the thrips populations was approximately 2 females to 1 male, except for NL1 (7:1), US1 (5:1), and JA (4:1).

Tospovirus isolates and test plants

In this study, the Brazilian TSWV isolate BR-01 (de Ávila *et al.*, 1990) and the INSV isolate NL-07 (de Ávila *et al.*, 1992) were used. TSWV and INSV were maintained by thrips inoculation of *Datura stramonium* L. and *Nicotiana benthamiana* Domin. plants, respectively. To acquire the virus, larvae were fed on TSWV-infected *D. stramonium*, *Impatiens* sp. or *N. benthamiana* plants, or on INSV-infected *Impatiens* sp. or *N. benthamiana* plants. These plants were mechanically inoculated on the first two true leaves with extracts from thrips-inoculated plants and kept in a greenhouse at approximately 22°C (16/8 h of light/dark).

Tospovirus detection by enzyme-linked immunosorbent assay (ELISA)

The viral nucleocapsid protein (N) content in leaves used as virus source, was determined with the double antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977; Resende *et al.*, 1991). Extracts were prepared by grinding small pieces of leaf tissue at a ratio 1:30 in PBS-T (0.14 M NaCl, 1 mM KH_2PO_4, 8 mM Na_2HPO_4, 2.5 mM KCl and 0.05% Tween-20). Extracts from noninfected plants were used as controls. Individual thrips were analysed for their TSWV-N protein content by a cocktail ELISA followed by enzyme amplification as described previously (Chapter 2). Polyclonal antisera raised against the N-protein of the BR-01 and NL-07 isolates were used in the DAS and cocktail-ELISA (Chapter 2). ELISA readings were corrected for blanks read from wells containing only sample buffer in the incubation step. Readings higher than the average noninfected controls, either from plants or thrips, plus three times their s.d. were considered positive, those with lower readings negative.

Handling thrips in transmission experiments

To obtain cohorts of equally aged larvae, fresh bean pods were placed in *F. occidentalis* colonies for egg oviposition. After 24 h, infesting thrips were removed and the pods incubated at 27°C. All larvae, up to 4-h-old, which emerged from the pods, were maintained in Tashiro cages (Tashiro, 1967). Thrips larvae were given an acquisition access period (AAP) on systemically infected leaves with comparable N-protein contents

(determined by ELISA). The leaves used as virus source were cut into pieces and randomly divided over the cages. Similarly sized groups of larvae were caged on noninfected plant material as control treatments. Before and after AAP, thrips were transferred to virus free plant material, and reared until adult emergence. The experiments were carried out at 25 ± 0.5°C. Each adult thrips was tested for its transmission ability on leaf disks (13 mm in diameter) of *Petunia x hybrida* cv. 'Polo Blauw' as previously described (Wijkamp and Peters, 1993) for three successive inoculation access periods (IAP) of 48 h. After each inoculation access period (IAP), the leaf disks were incubated on water for 3 days at 27 ± 0.5°C to develop local lesions. The efficiency was calculated as the percentage of infected leaf disks.

TSWV transmission by different F. occidentalis *populations*

Newly hatched larvae were placed on TSWV-infected *D. stramonium* for 24 h. After this AAP, the thrips completed their larval and pupal development on noninfected *D. stramonium* material. Directly after becoming adult, thrips were individually tested for their transmission ability. Adults of ten *F. occidentalis* populations were, after this testing, stored at -70°C and later assayed in cocktail-ELISA followed by enzyme amplification to determine the percentage of ELISA-positive thrips. Transmission and ELISA experiments were executed with 27 to 90 adults per population (Fig. 1).

Virus acquisition by first and second instar larvae of F. occidentalis *populations*

Newly hatched first instar (L1) larvae, 36-h-old larvae (being a mixture of L1s and second instars (L2s)), and L2 larvae (72-h-old) of the *F. occidentalis* populations IS2, NL3, and US2 were placed for a 4-h AAP on TSWV- or INSV-infected leaf material of *N. benthamiana* plants. Before and after this AAP, the thrips were kept on virus free *D. stramonium* plant material. The adult transmission efficiency was calculated using the petunia leaf disk assay (Wijkamp and Peters, 1993). In total, 44 to 113 thrips were used per treatment (Fig. 2).

Virus acquisition and transmission by F. occidentalis *from and to different plant species*

Up to 4-h-old larvae of the populations IS2, NL3 and US2 were placed for 24 h on TSWV-infected leaf material of *D. stramonium* and *Impatiens* sp. The selected leaves had approximately the same virus contents, as determined by ELISA. After this AAP, the thrips were maintained on leaves from noninfected plants of the species on which the virus was ingested. When becoming adults, their ability to transmit TSWV was individually tested in a 48-h IAP on leaf disks of the species on which the larvae were maintained, and in two IAPs of 48 h on petunia leaf disks. To randomise the effect of ageing of thrips on the transmission, these IAPs were organised in a Latin square design. Infection was either demonstrated by local lesions on petunia disks or by ELISA when disks of other plant

species, which only develop systemic symptoms, were used. The transmission efficiency was expressed by the percentage of disks infected. The ELISA readings of the leaf disks infected by the different *F. occidentalis* populations were compared. Leaf disks of noninfected plants of *D. stramonium* and *Impatiens* sp. plants served as controls. Results were obtained with 43, 33, and 61 adults on *D. stramonium* leaf disks, and 20, 13, and 29 adults on *Impatiens* sp. disks, of the populations IS2, NL3, and US2, respectively.

To estimate the amount of TSWV-N protein ingested, approximately 18 larvae of the populations IS2, NL3 and US2 were randomly collected after an 8-h-AAP and stored at -70°C prior to ELISA. The ELISA readings were analysed using Duncan's multiple range test with a STATGRAPHICS 6.0 PLUS computer program (Rijpkema, 1993; Schulman, 1992).

Results

Variation in TSWV transmission efficiencies between distinct F. occidentalis *populations*

Fourteen populations of *F. occidentalis*, originating from various geographical regions (Table 1), were compared for their ability to transmit TSWV. These populations may transmit virus with different efficiencies as they came from different biological niches. The efficiency by which the adults transmitted TSWV to petunia leaf disks and the percentage of ELISA-positive adults were determined (Figs. 1a and b).

Forty-eight percent of the adults of the Dutch reference strain NL3 transmitted TSWV. Approximately similar efficiencies were found for the populations collected in France (FR), Israel (IS1), Japan (JA) and New Zealand (NZ), and the two other Dutch populations (NL1, NL2). Two American populations (US1, US2) and a Dutch population (NL5) transmitted the virus at a considerable lower efficiency than NL3. In contrast, four populations (IS2, DK, IT and NL4) transmitted TSWV with an efficiency, which was approximately 1.5 times higher than that of NL3. IS2 was, with an efficiency of 75%, the population which transmitted the virus most efficiently. These results indicate that the transmission ability does not only differ between thrips populations from different geographic regions, but also between populations collected from the same region, i.e. Israel (cf. IS1 and IS2) and the Netherlands (cf. NL3, NL4 and NL5).

The percentage of ELISA-positive adults was always a factor of 1.2 to 2.7 higher than the transmission efficiency. These results showed that transmission efficiency and percentage ELISA- positive thrips can be used as adequate parameters to differentiate *F. occidentalis* populations. In further studies, the transmission efficiency by adult thrips was the most useful parameter instead of the time-consuming determination of percentage of ELISA-positive adults to characterise tospovirus transmission capacities of *F. occidentalis* populations.

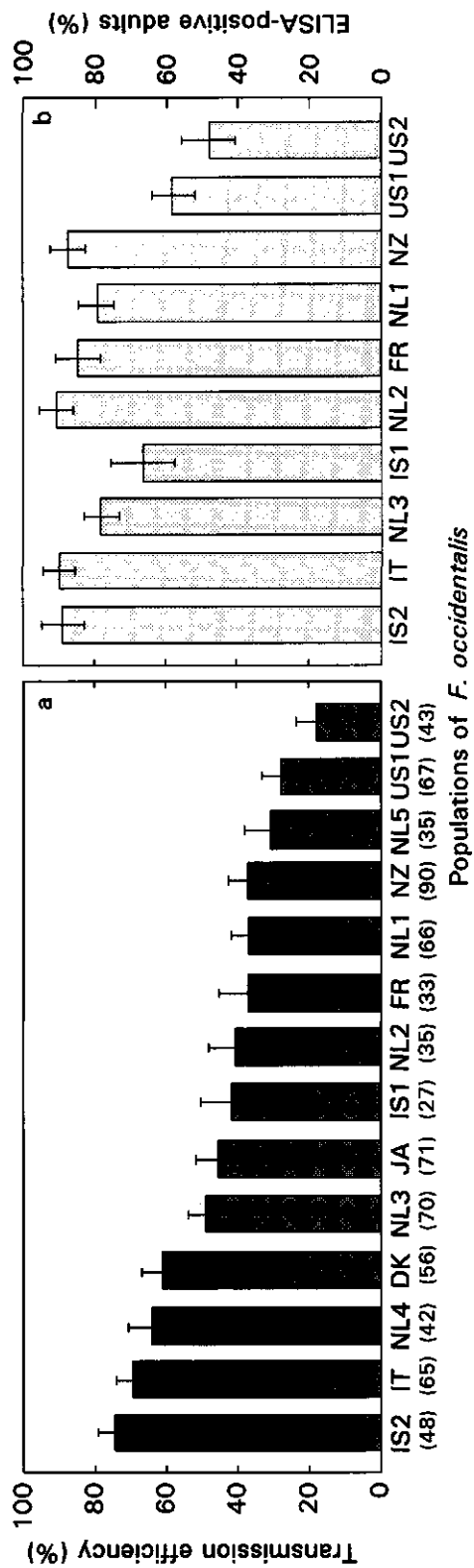

Figure 1. First cumulative tomato spotted wilt tospovirus (TSWV) transmission efficiency (%) by adult male and female *Frankliniella occidentalis* determined after each successive inoculation access period (IAP) of 2 days after adult emergence. This efficiency was determined for the total number of living thrips at each IAP (dark grey bars), and for the number of living thrips at day 6 (light grey bars) ($n = 35$ and $n = 63$ for males and females at day 6, respectively). Vertical bars indicate s.e.'s.

TSWV and INSV acquisition by first and second instar larvae of three F. occidentalis *populations*

Previous studies showed that only the first instar larvae of the *F. occidentalis* population NL3 could acquire TSWV (Chapter 2). To test whether this observation was a general phenomenon of *F. occidentalis*, the ability to acquire TSWV was also determined for the first and second instar larvae of the populations IS2 and US2. Both populations were chosen because they differed strongly in their ability to transmit TSWV (Fig. 1a).

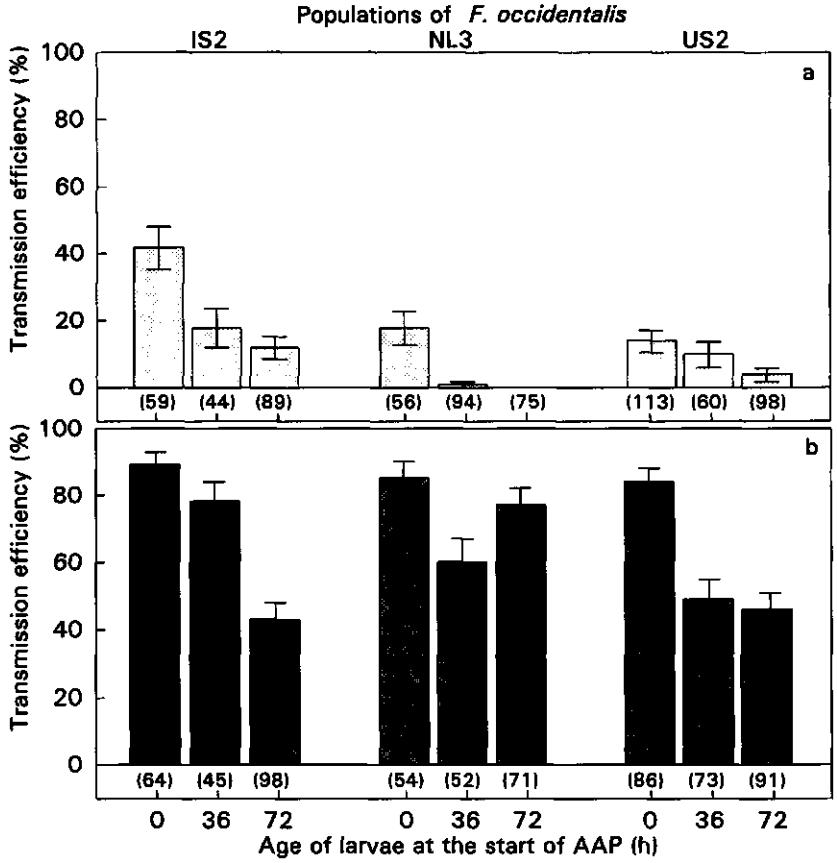

Figure 2. (a) Tomato spotted wilt virus (TSWV)- and (b) impatiens necrotic spot virus (INSV)-transmission after a 4-h-acquisition access period (AAP) by newly hatched (L1), 36 h (L1 + L2) and 72 -h-old larvae (L2) of *Frankliniella occidentalis* populations from Israel (IS2), the Netherlands (NL3) and United States (US2). Averages ± s.e. are presented. Values in parentheses represent the number of thrips tested.

Adults of the NL3 population failed to transmit when 72-h-old L2 larvae ingested TSWV (Fig. 2a), confirming our earlier results (Chapter 2). In the present study, TSWV ingestion by 72-h-old L2s of the populations IS2 and US2 resulted in an adult TSWV transmission efficiency of 12 and 4%, respectively, showing that transmission efficiencies were considerably lower than when first instar larvae were allowed to ingest TSWV.

To study whether tospovirus acquisition is, like TSWV, restricted to first instar larvae, a second tospovirus species, INSV, was included in these experiments. INSV was more efficiently transmitted by *F. occidentalis* than TSWV, conform previous studies (Wijkamp and Peters, 1993; Wijkamp *et al.*, 1995). Adults of all three populations transmitted INSV at efficiencies of approximate 85%, after ingesting the virus as newly hatched larvae (Fig. 2b). The 72-h-old L2s of all three populations were able to acquire INSV, including the NL3 population, and transmitted this virus with efficiencies ranging between 43% (IS2) and 77% (NL3). These results also show that transmission of INSV by the different *F. occidentalis* populations is affected by the age of virus-ingesting larvae and that these populations have distinct capacities to transmit INSV.

Ingestion of TSWV by first instar larvae of the IS2, NL3 and US2 populations was analysed after they had fed for 8 h on TSWV-infected *D. stramonium* and *Impatiens* sp. leaf material (Table 2). The results showed that TSWV ingestion did not differ significantly for the three *F. occidentalis* populations tested, according to Duncan's multiple range test ($P<0.05$).

Table 2. Ingestion of tomato spotted wilt virus (TSWV) in 8 h by newly hatched larvae of three populations of *Frankliniella occidentalis* on *Datura stramonium* and *Impatiens* sp.

	Plant species					
Populations of	*Datura stramonium*			*Impatiens* sp.		
F. occidentalis	n[1]	pos[2]	ELISA readings[3]	n[1]	pos[2]	ELISA readings[3]
IS2	18	18	0.126 ± 0.044[4a]	18	15	0.093 ± 0.022[4a]
NL3	15	14	0.146 ± 0.023[a]	17	7	0.072 ± 0.019[a]
US2	17	17	0.112 ± 0.019[a]	19	9	0.031 ± 0.002[a]

[1] Number of thrips tested in ELISA; [2] Number of tested thrips which were TSWV-positive in ELISA at 490 nm. Thrips were denoted positive as their readings were above the threshold reading, which was calculated as the average of virus-free thrips + 3 times the s.d. (ranging from 0.019 to 0.021 in this experiment); [3] ELISA readings, at 405 nm, denoted as the average ± s.e.; [4] ELISA readings within a column followed by the same letter are not significantly different according to Duncan's multiple range test ($P<0.05$).

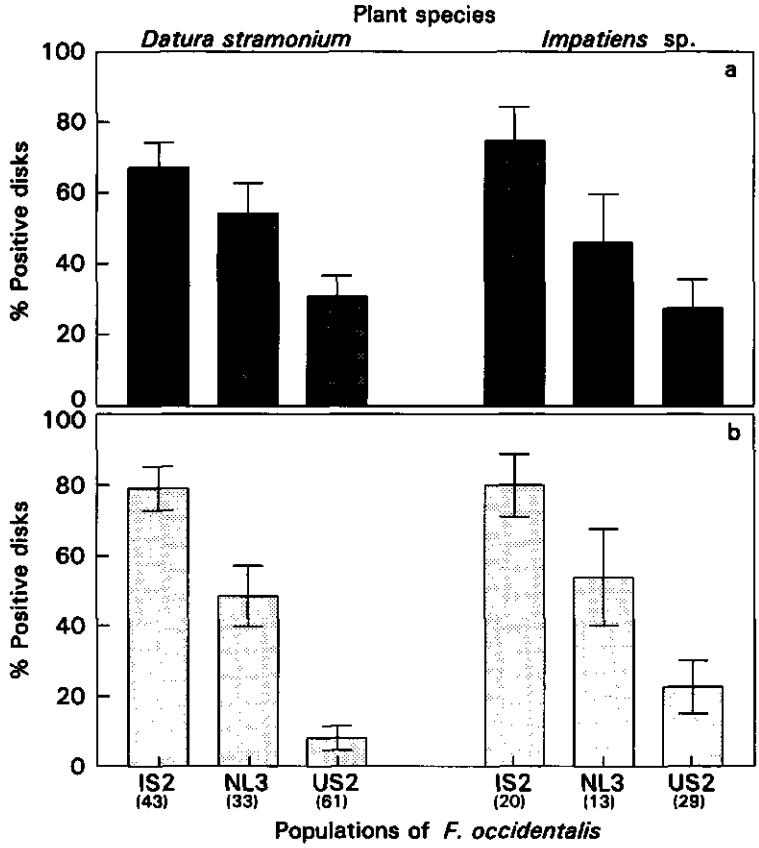

Figure 3. Tomato spotted wilt virus (TSWV)-transmission efficiency (± s.e.) after a 24-h-acquisition access period (AAP) on TSWV-infected plant material of *Datura stramonium* or *Impatiens* sp. by different populations of *Frankliniella occidentalis*. After the AAP, the thrips were reared on virus free plant material of the same host species as the virus source. The adult thrips were tested on the disks from the plant species on which they acquired TSWV (a) or on petunia leaf disks (b). Values in parentheses represent the number of thrips tested.

Effect of plant species on TSWV acquisition and transmission.

D. stramonium and *Impatiens* sp. plants were used to test possible host plant effects on the acquisition and transmission of TSWV by the *F. occidentalis* populations IS2, NL3 and US2. Each population transmitted the virus at an almost similar efficiency to the leaf disks of petunia, *D. stramonium* and *Impatiens* sp. (Fig. 3). Hence, it can be concluded that the efficiency at which the different *F. occidentalis* populations transmit tospovirus is not affected by the plant species used, as tested so far. In addition, the development from L1 to adult did not differ between these three populations when they were reared on *D. stramonium* or *Impatiens* sp. plants (results not shown).

The ELISA readings of the leaf disks infected by viruliferous adults of the populations IS2, NL3, and US2 differed, however, considerably (Duncan's multiple range test, $P<0.05$; Table 3). Leaf disks of *D. stramonium* inoculated by viruliferous adults of IS2 had considerably higher ELISA readings than those inoculated by NL3 adults. In addition, infected disks of *D. stramonium* and *Impatiens* sp. by thrips of US2 population had significantly lower ELISA readings, implying a lower virus content, than disks infected by adults of the IS2 and NL3 population.

Table 3. Enzyme-linked immunosorbent assay (ELISA) readings at 405 nm of tomato spotted wilt virus (TSWV)-infected leaf disks of *Datura stramonium* and *Impatiens* sp. Three *Frankliniella occidentalis* populations were used to inoculate the leaf disks.

Populations of	Plant species	
F. occidentalis	*Datura stramonium*	*Impatiens* sp.
IS2	(29)[1] 0.658 ± 0.087[2a]	(15)[1] 1.108 ± 0.061[2a]
NL3	(18) 0.384 ± 0.091[b]	(6) 1.035 ± 0.071[a]
US2	(19) 0.061 ± 0.008[c]	(8) 0.698 ± 0.113[b]

[1] Number of disks tested is indicated in parentheses; [2] ELISA readings denoted as the average ± s.e. Readings within a column followed by the same letter are not significantly different according to Duncan's multiple range test ($P<0.05$).

Discussion

These studies demonstrated that all *F. occidentalis* populations, collected from fourteen different regions, were capable to transmit TSWV. However, the efficiencies, expressed as the percentage transmitting adults of both sexes, by which these populations transmitted this virus varied considerably (Figs. 1a and b, 2a and b); ranging from 18% (population US2) to 75% (population IS2). Rearing of these populations under identical conditions for at least two-and-a-half years on bean pods apparently did not outgrow these different transmission competencies. Based on these results and the constant efficiency by which the NL3 reference population has transmitted TSWV over a period of six years (Chapter 2; Wijkamp and Peters, 1993; Wijkamp *et al.*, 1993 and 1995), it can be concluded that the tospovirus transmission efficiency is a stable character for each thrips population. Hence, the distinct tospovirus transmission competencies of populations are likely the result of their genetic adaptation to their original biological niche.

The variation in the ability to transmit virus may be due to dissimilar midgut and salivary gland properties between the *F. occidentalis* populations, affecting the efficiency of acquisition and/or replication and transmission of the virus. Differences in the midgut receptors, the activity of digestive enzymes in the gut affecting the infectivity of the virus, and dissemination barriers may be some of the factors regulating the acquisition and release of the virus by and from the gut cells. At the level of salivary glands, a barrier may exist in the penetration of the virus in the gland cells. Differences in the replication rate or release of virus in the saliva may be another factor as has been described for other propagative viruses (Ammar, 1994). The study presented here does not allow discrimination between any of these factors.

As different *F. occidentalis* populations have distinct tospovirus transmission competencies, the question arises whether we can denote some of these populations as different 'biotypes'. Broadly speaking, the term biotype is an intraspecific category for a biological attribute, which refers to insect populations of similar genetic composition (Saxena and Barrion, 1987). Biotypes can be classified in e.g. aggressiveness towards resistant or susceptible varieties of crop plants and/or disease vector capabilities, like the transmission ability of tospoviruses. According to the results described in this paper, we might classify some *F. occidentalis* populations, which transmit tospovirus with dissimilar efficiencies, as different biotypes. Examples of this are the populations IS2, NL3 and US2. Various *F. occidentalis* populations used in this paper, were studied by de Kogel *et al.* (1997c) for their performance on cucumber accessions with different levels of resistance. They concluded that the reproduction rate of each population could be used as a criterion for differentiating *F. occidentalis* populations, and considered e.g. the populations of NZ and NL1 as two distinct populations. As the *F. occidentalis* populations NZ and NL1 did not differ significantly in TSWV transmission (Fig. 1a), we can conclude that differences between populations greatly depend on the biological characteristics studied. Future studies on the populations, including genetic analysis, are needed to describe the interpopulation variation in tospovirus transmission as biotypic variation.

Several studies on biotypic variation in insect species have been reported in relation to the transmission of other circulative plant viruses (Sylvester, 1980). Detailed studies with whitefly-transmitted geminiviruses showed that differences in transmission efficiency and virus specificity were associated mostly with differences in the epitope profiles of the coat protein of these viruses (McGrath and Harrison, 1995). Likewise, differences between *F. occidentalis* populations in transmitting two tospoviruses (Figs. 2a and b) might be associated with distinct structural features of the surface (glyco)proteins of tospoviruses. Bedford *et al.* (1994) reported that differences in geminivirus transmission by distinct whitefly, *Bemisia tabaci*, biotypes (Bedford *et al.*, 1992 and 1993; Brown *et al.*, 1992; Byrne and Devonshire, 1993, Perring *et al.*, 1993a), which are also considered to be

different species (Bartlett and Gawell, 1993; Bedford *et al.*, 1993; Campbell *et al.*, 1993; Perring *et al.*, 1993a and b), resulted from virus distribution in the plant and feeding (ingestion) behaviour. Since studies in this paper were executed with virus sources that contained similar virus contents and that were randomly divided over the Tashiro cages, the variation found in transmission efficiency between the different *F. occidentalis* populations cannot be attributed to differences in virus distribution or concentration. Also, the amount of TSWV ingested by the different *F. occidentalis* populations was comparable (Table 2), ruling out an ingestion effect on the obtained transmission variation.

Differences in transmission efficiencies by *F. occidentalis* populations were greatly influenced by the age of larvae when feeding on infected material, and by the tospovirus species used (Figs. 2a and b). First and second instar larvae of the *F. occidentalis* populations IS2 and US2 acquired both TSWV and INSV. Likewise, INSV transmission took place after ingestion by both instar larvae of the NL3 population, whereas the second instars of this population failed to acquire TSWV. It can, therefore, be concluded that the ability of larvae to acquire tospovirus depends on both the *F. occidentalis* population and the tospovirus species used. The dissimilarities in TSWV and INSV acquisition by the second instar larvae of the NL3 population, may be explained by e.g. the presence of different receptors in the thrips for both tospovirus species, or alternatively, by the activity of digestive/proteolytic enzymes in the midgut having different effects on the two species analysed.

TSWV transmission efficiencies by *F. occidentalis* populations were not affected by the plant species used as virus source and test plants, as shown for *D. stramonium* and *Impatiens* sp. (Figs. 3a and b). However, the virus content in leaf disks of these species infected by viruliferous thrips differed. The disks infected by viruliferous IS2 adults, the most efficient transmitter, had the highest virus content, whereas the lowest amount of TSWV antigen was found in leaf disks infected by the poorest transmitter, US2 (Table 3). These data suggest that the *F. occidentalis* populations may inject different amounts of TSWV in disks, resulting in different virus contents. Although not very likely, it cannot be ruled out that some selection might occur during the virus multiplication in the vector, resulting in virus variants with different replication abilities in plants. Future research has to verify whether whole plants, as found for leaf disks, are also poorly infected by the *F. occidentalis* population US2, and efficiently by IS2.

In view of the distinct differences in tospovirus transmission efficiencies between *F. occidentalis* populations, improved understanding of tospovirus-thrips interactions need detailed analysis not only at organism but also at population level. Also, general conclusions on tospovirus spread should be based on dynamics of a number of vector populations, to circumvent inaccurate statements. To date, tospovirus control methods are focussed on early detection of tospovirus and thrips vectors, and on the prevention and elimination of

virus introduction and spread in the crop. The variation in tospovirus transmission by several populations of one thrips species makes prediction of virus damage very difficult. In our view, the currently applied integrated tospovirus management should be focussed on the worst case scenario, i.e. the most efficient vector population, in which adequate (chemical) treatments are needed when a vector species is identified in a production area, irrespective of the transmission competency of the population.

Acknowledgements

This research was financed by fellowship No. ABI22.2704, of the Foundation of Dutch Technology (STW) of the Netherlands Organisation for Scientific Research (NWO). We thank Novartis B. V., Enkhuizen, the Netherlands, for supplying us with *Petunia x hybrida* cv. 'Polo Blauw' seeds. We also like to thank M. Klein for his interest and useful discussions during the experiments. We are most grateful to all researchers who supplied us samples of different *F. occidentalis* populations (Table 1), and to W.J. de Kogel for rearing and maintaining them.

Chapter 4

Differences in tomato spotted wilt virus vector competency between males and females of *Frankliniella occidentalis**

Abstract

Possible differences in tomato spotted wilt virus (TSWV) transmission vector competency between *Frankliniella occidentalis* males and females were investigated. For the Dutch reference population NL3 the transmission efficiency was notably higher for adult males (57%) than for females (32%). Moreover, viruliferous males of this population were able to repeatedly transmit virus within six days after adult emergence, whereas significantly less viruliferous females were able to transmit more than a single time. For both sexes, the transmission efficiency dropped with age, simultaneously with decreasing consumption rate. The higher vector efficiency for males was verified to be a general phenomenon as this feature was also found for thirteen other *F. occidentalis* populations, which originated from distinct geographic regions.

*This chapter has been submitted for publication to Entomologia Experimentalis et Applicata as: van de Wetering, F., M. van der Hoek, R. Goldbach, and D. Peters. Differences in tomato spotted wilt virus vector competency between males and females of *Frankliniella occidentalis*.

Introduction

To date tomato spotted wilt tospovirus (TSWV) belongs to the ten most devastating plant viruses world-wide. TSWV is exclusively transmitted by various thrips species (Thysanoptera: Thripidae). At present, 5000 thrips species are recognised (Mound, 1996), of which only eight species are known to transmit tospoviruses (Webb *et al.*, 1998; Wijkamp *et al.*, 1995). Of these, *Frankliniella occidentalis* (Pergande) is one of the most efficient transmitters of TSWV (Wijkamp *et al.*, 1995), and is considered a pest all over the world (Anonymous, 1989).

The thrips life cycle consists of six stages: egg, first instar larval (L1), second instar larval (L2), prepupal, pupal and adult stage. Only the L1s, L2s and adults are active feeders, and, hence, play a role in TSWV transmission. TSWV has to be acquired by young larvae (Chapter 2) and is transmitted by old L2s and adults (Wijkamp and Peters, 1993) after its replication and circulation in the thrips (Ullman *et al.*, 1993; Wijkamp *et al.*, 1993). Recent studies showed that adult males and females, from a population collected in the Netherlands (NL3), transmit TSWV with a different efficiency (Chapter 5), possibly due to a distinct feeding behaviour. Females are larger than males (Tommasini and Maini, 1995), have a reproductive function, and therefore ingest a higher quantity of food.

In this study, possible different vector competencies between adult males and females of *F. occidentalis* were investigated, as well as the effect of age. Most investigations were done with the Dutch reference population NL3, and part of the results obtained corroborated by comparing fourteen different *F. occidentalis* populations.

Materials and methods

Thrips cultures and tospovirus isolates

Virus-free colonies of *F. occidentalis* were reared on bean pods, *Phaseolus vulgaris* L. cv. 'Prelude', in addition of commercially available pollen and 10% sucrose solution, at 27°C and a daily photoperiod of 16 h. *F. occidentalis* populations were collected in Denmark (DK), France (FR), and Italy (IT) from bean, in Israel from strawberry (IS1) and mango (IS2), in Japan from chrysanthemum (JA), in the Netherlands from cucumber (NL1), chrysanthemum (NL2), bean (NL3), tomato (NL4), and rose (NL5), in New Zealand from egg plant (NZ), and in USA from gloxinia (US1) and chrysanthemum (US2) (de Kogel *et al*, 1997c). The NL3, NL4 and NL5 populations were cultured in our laboratory, whereas the other cultures were maintained for at least two-and-a-half years at the Centre for Plant Breeding and Reproduction Research (CPRO-DLO), Wageningen, the Netherlands, before this study was made. In the experiments described, the Brazilian TSWV isolate BR-01 (de Ávila *et al.*, 1990) was used. The isolate was maintained by thrips inoculation on *Datura stramonium* L. plants.

Enzyme-linked immunosorbent assay

The virus content in viruliferous thrips or infected plants was determined by enzyme-linked immunosorbent assay (ELISA). Used antisera raised against TSWV nucleocapsid protein (N) and a TSWV-nonstructural protein (NS_s) has been described previously by de Ávila *et al.* (1992) and Kormelink *et al.* (1991), respectively. The NS_s antiserum was cross-absorbed with acetone-washed powder of a mixture of noninfected thrips and *Spodoptera frugiperda* to eliminate background in ELISA due to non-specific reactions (Hampton *et al.*, 1990).

Extracts of leaf tissue were tested in a double antibody sandwich (DAS)-ELISA format (Clark and Adams, 1977; Resende *et al.*, 1991). These extracts were prepared by grinding leaf material with PBS-T (PBS with 0.05% Tween-20) in a ratio 1:30 (w/v). Leaf extracts from noninfected plants were used as controls. Absorbance was measured at 405 nm using an EL 312 ELISA-reader (Bio-Tek Instruments, Greiner BV, Alphen aan de Rijn, the Netherlands). Wells containing only PBS-T in the sample incubation round were used as blanks.

The virus content of thrips was analysed by cocktail-ELISA (Resende *et al.*, 1991) and by antigen-coated plate (ACP) ELISA both followed by enzyme amplification. Individual thrips were triturated with a micropestle in 100 μl of PBS-T containing 2% polyvinylpyrrolidone [M_w about 44,000] and 0.2% ovalbumin (sample buffer). Each suspension was divided in two equal portions. One was tested with anti-N, the other with anti-NS_s sera. A slightly modified cocktail ELISA format was performed using anti-N sera (Chapter 2). The NS_s protein content in thrips was determined by ACP-ELISA, in a format described previously (Wijkamp *et al.*, 1993). Subjected to cocktail and ACP-ELISA, the enzyme amplification reaction (van den Heuvel and Peters, 1989) was conducted with some modifications (Chapter 2). Subsequently, the absorbance of each well was read at 490 nm. The cocktail- and ACP-ELISAs were conducted at the same time, to prevent differences in data due to different conditions in time. In the ELISA formats, noninfected thrips were used as controls and a dilution series of N-protein purified from TSWV infected *Nicotiana benthamiana* (cocktail-ELISA), and an extract of *S. frugiperda* cells (ACP-ELISA) were used as standards. The readings were corrected for blank values that were read for wells that only contained sample buffer in the sample incubation round. All readings above the average value of noninfected thrips plus 3 times s.d. were considered ELISA-positive.

TSWV transmission experiments

Virus sources, required for virus acquisition, were obtained by mechanical inoculation of the first true leaves of *D. stramonium* L. plants with TSWV, using inocula that consisted of thrips-inoculated material ground at a 1:5 ratio with inoculation buffer (0.02 M NaH_2PO_4, 0.08 M Na_2HPO_4, and 0.1 M Na_2SO_3). Systemically infected leaves were tested for their TSWV-N content in DAS-ELISA, and those with similar contents were selected as virus

source. Selected leaves were cut into pieces and divided randomly over Tashiro cages (Tashiro, 1967). Larvae, up to 4 h old, were enclosed in these cages for an acquisition access period (AAP) of 24 h. After this AAP, the thrips were maintained on noninfected *D. stramonium* leaves until the adult stage. Subsequently, the sex of each adult was determined and the thrips were individually tested for their ability to transmit the virus, using petunia (*Petunia x hybrida* cv. 'Polo Blauw') local lesion assay (Wijkamp and Peters, 1993). After three days, the petunia disks were scored for TSWV symptoms, which appeared as black to brown local lesions. The transmission efficiency was determined as the percentage of disks developing symptoms, corresponding with the percentage of viruliferous adults.

In the first type of experiments, males and females of the Dutch reference population NL3 were randomly divided into four groups immediately after adult emergence. The adults of the first group were individually maintained until death on petunia leaf disks. The survival of each individual was checked daily. At the end of this experiment, the development time from L1 to pupa (days) and the adult lifetime (days) were determined, and differences between parameters of males and females were analysed using the Student-*t*-test (Rijpkema, 1993; Schulman, 1992). Thrips from the second, third and fourth group were put on petunia leaf disks for one inoculation access period (IAP), two IAPs, or three IAPs of 2 days, respectively. After the final IAP, the thrips were immediately stored at -80°C prior to ELISA. The first cumulative transmission efficiency, defined as the sum of those thrips that made a first transmission, was determined after each IAP. Differences in transmission efficiencies between males and females were analysed using Chi-squared statistics (Rijpkema, 1993; Schulman, 1992). When ELISA was followed by enzyme amplification, the percentage of thrips containing TSWV-N or TSWV- NS_s and the average absorbance value (read at 490 nm) were determined.

In the second type of experiments, adult males and females of fourteen *F. occidentalis* populations, collected from different regions all over the world (Chapter 3; de Kogel *et al.*, 1997c), were individually analysed for their ability to transmit TSWV in three successive IAPs of 2 days on petunia leaf disks. The transmission efficiency of males and females was determined for each population and compared using Chi-squared statistics (Rijpkema, 1993; Schulman, 1992). Three populations, i.e. IS2 (Israel), NL3 (the Netherlands), and US2 (USA), were selected to determine the virus titer (TSWV-N) per sex and to combine transmission and ELISA data for both sexes. Absorbance readings (read at 490 nm) between ELISA-positive transmitters and nontransmitters were analysed with the Student-*t*-test (Rijpkema, 1993; Schulman, 1992).

Determination of food ingestion

At the same time when the first transmission experiments were executed, the amounts of food ingested by males and females of *F. occidentalis* population NL3 were determined. Noninfected adults were fed on TSWV-infected *D. stramonium* when they were 0-2, 2-4 or 4-6 days old. After these feeding periods, thrips were analysed for the TSWV content in ELISA using anti-N and anti-NS_s-sera after storage at -80°C. The percentage of ELISA-positive adults, containing TSWV-N or TSWV-NS_s, as well as their average absorbance reading at 490 nm, were determined.

Results

TSWV transmission by ageing males and females of F. occidentalis

To optimise comparisons, age-dependent differences between males and females of the *F. occidentalis* population NL3 with respect to their development time and adult lifetime were determined. For both males and females the development from L1 to pupae took 9.9 days (Table 1). However, the average lifetime of adult females was twice as long as for males, 12.8 and 6.4 days, respectively. For both sexes, the mortality was low in the first six days after adult emergence; hence this period was used to study the effect of ageing on TSWV transmission.

Table 1. Average time ± s.e. (days) to develop from newly hatched larvae to pupae and their lifetime after adult emergence of *Frankliniella occidentalis* males and females at 25°C.

	n[1]	Total development Time (larvae-pupae) on *Datura stramonium*	Adult life-time on petunia
Male	44	9.9 ± 0.2	6.4 ± 0.3*[2]
Female	66	9.9 ± 0.1	12.8 ± 0.7

[1] Number of thrips tested; [2] Asterisk indicate a significant difference within a column at $P<0.001$, using Student-*t*-test.

The efficiency by which males and females transmitted TSWV was recorded by their first transmissions for all living thrips at each IAP, and for those still alive after the three IAPs (day 6). The outcome of both methods was similar (Fig. 1), indicating that both are suitable to determine first cumulative tospovirus transmission efficiencies. The majority of viruliferous thrips already transmitted in the first IAP. Most, if not all, viruliferous adult

thrips were able to transmit TSWV in the second IAP after emergence. Males transmitted TSWV more efficiently than females, reaching a maximal virus transmission efficiency of 57%, whereas females transmitted TSWV with 32% efficiency.

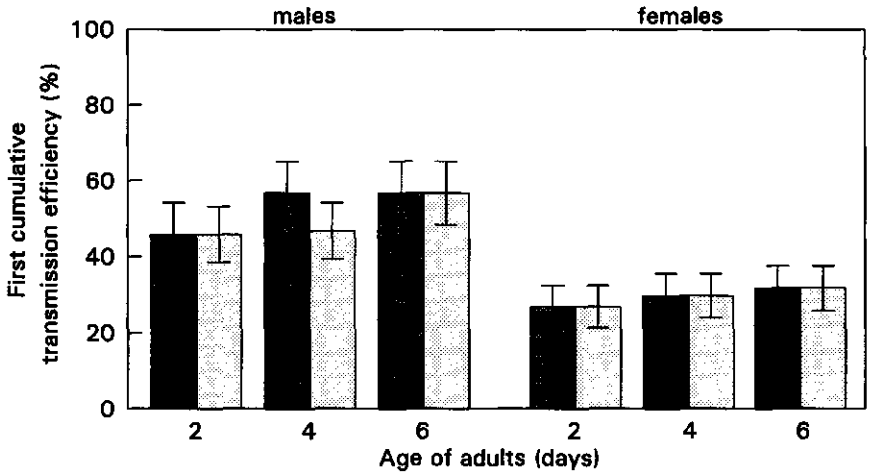

Figure 1. First cumulative tomato spotted wilt tospovirus (TSWV) transmission efficiency (%) by adult male and female *Frankliniella occidentalis* determined after each successive inoculation access period (IAP) of 2 days after adult emergence. This efficiency was determined for the total number of living thrips at each IAP (dark grey bars), and for the number of living thrips at day 6 (light grey bars) ($n = 35$ and $n = 63$ for males and females at day 6, respectively). Vertical bars indicate s.e.'s.

After each IAP, a portion of the adult thrips was collected and each thrips was individually analysed for its viral antigen content. Higher percentages of males (68%) than females (60%) became ELISA (N)-positive (Fig. 2a). The percentage sero-(N)-positive thrips matched the trend of first cumulative transmission efficiencies (Fig. 1), as both percentages increased with age. Maximum levels were obtained when the adults were four days old. Also, the amounts of TSWV increased considerably during the first days after adult emergence (Fig. 2b). In addition, the amount of NS_s protein, a protein that is only present during replication of the viral genome in the thrips (Kormelink *et al.*, 1991; Wijkamp *et al.*, 1993), and percentage of NS_s-positive thrips decreased when thrips aged (Figs. 2a and b). Hence, it is concluded that virus replication in the thrips occurs predominantly within the first days after adult emergence, reaching such high virus (TSWV-N) titers in four day old thrips that at that time the virus is transmitted with a maximal efficiency (Figs. 1, 2a and b).

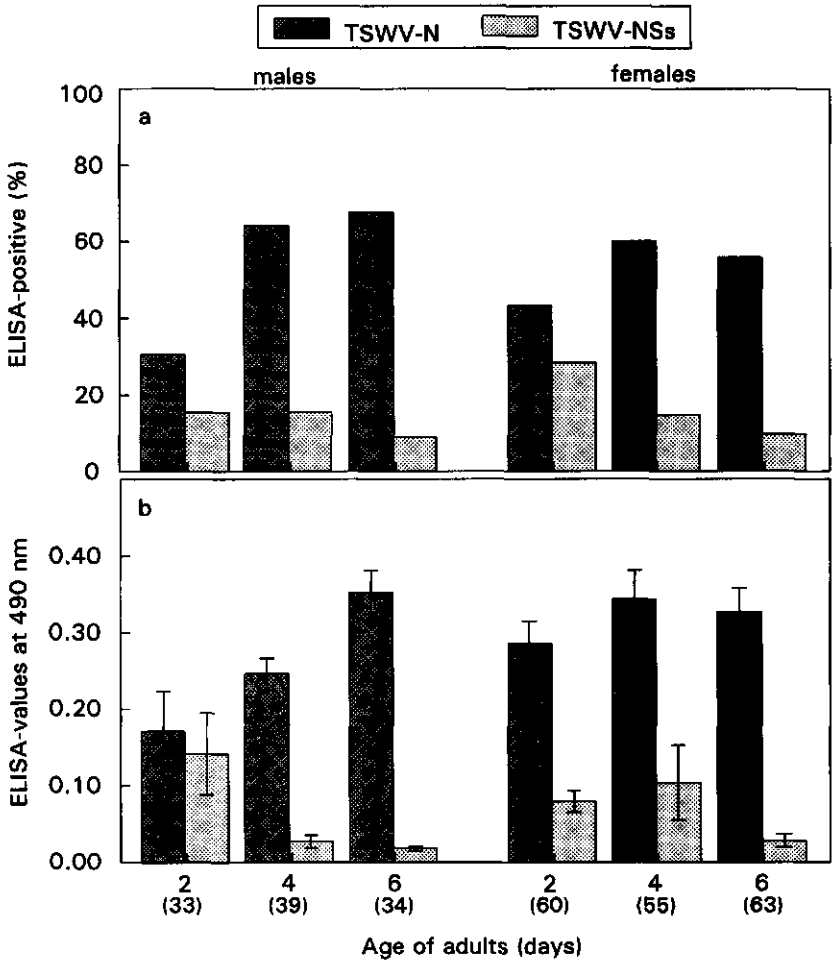

Figure 2. Detection of acquired tomato spotted wilt tospovirus (TSWV) in ageing adult male and female *Frankliniella occidentalis* by enzyme-linked immunosorbent assay (ELISA), using antisera against the TSWV-nucleocapsid protein (N) and a TSWV-nonstructural protein (NS_s). The percentage ELISA-positive adults (a) and average (± s.e.) ELISA values at 490 nm (b) were determined. All readings above the average value of noninfected thrips + 3 times s.d. were considered ELISA-positive. Numbers of thrips are indicated in parentheses. Vertical bars indicate s.e.'s.

Distinct TSWV transmission capacities by males and females

To quantify TSWV transmission competencies of *F. occidentalis* males and females separately, the frequency of transmissions in three successive IAPs of 2 days and the percentage viruliferous males and females per IAP were determined.

Significant differences were observed in the frequency by which viruliferous males and females transmitted during these three successive IAPs (Fig. 3a). A significant higher percentage of viruliferous males transmitted TSWV three times, whereas significantly less females infected the petunia leaf disks more than once. Furthermore, the transmission efficiency was found to decrease with age (Fig. 3b), males always retaining higher efficiencies than females during the second and third IAP.

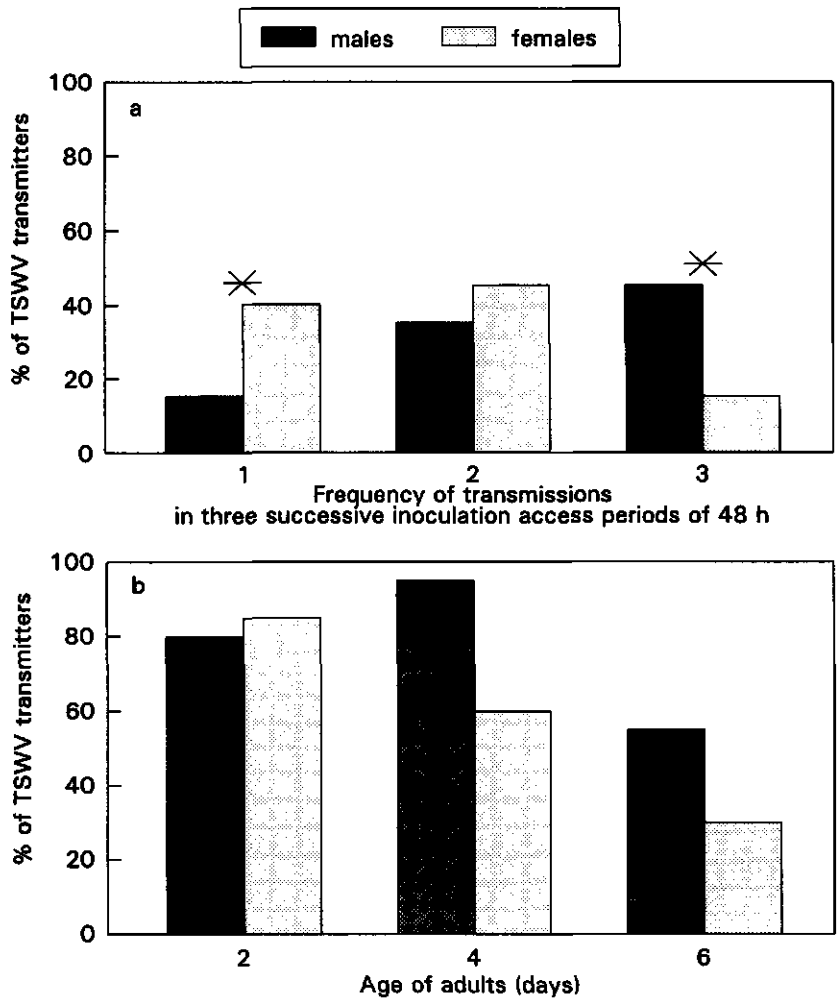

Figure 3. Percentage of adult male or female *Frankliniella occidentalis* which transmitted tomato spotted wilt tospovirus (TSWV) once, twice or three times during three successive inoculation access periods (IAPs) of 48h (a), and which transmitted TSWV per IAP of 48h (b). Asterisks indicate significant differences as analysed with Chi-squared statistics at $P<0.005$.

Food ingestion by males and females of increasing age

A possible relationship between the amount of food ingested and the efficiency of TSWV transmission was investigated, as higher consumption rates might favour virus transmission due to more saliva (and therefore TSWV) egestion. Previous studies showed that virus ingestion from TSWV-infected *D. stramonium* leaves could be correlated with food ingestion by larvae (Chapter 2). Hence, the same method was used to determine the food ingestion by males and females of increasing age. In the three feeding periods, both sexes ingested readily detectable amounts of TSWV, resulting in up to 100% of ELISA-positive thrips (Fig. 4a). The TSWV-N content decreased in ageing thrips (Fig. 4b), matching TSWV transmission per IAP (Fig. 3b).

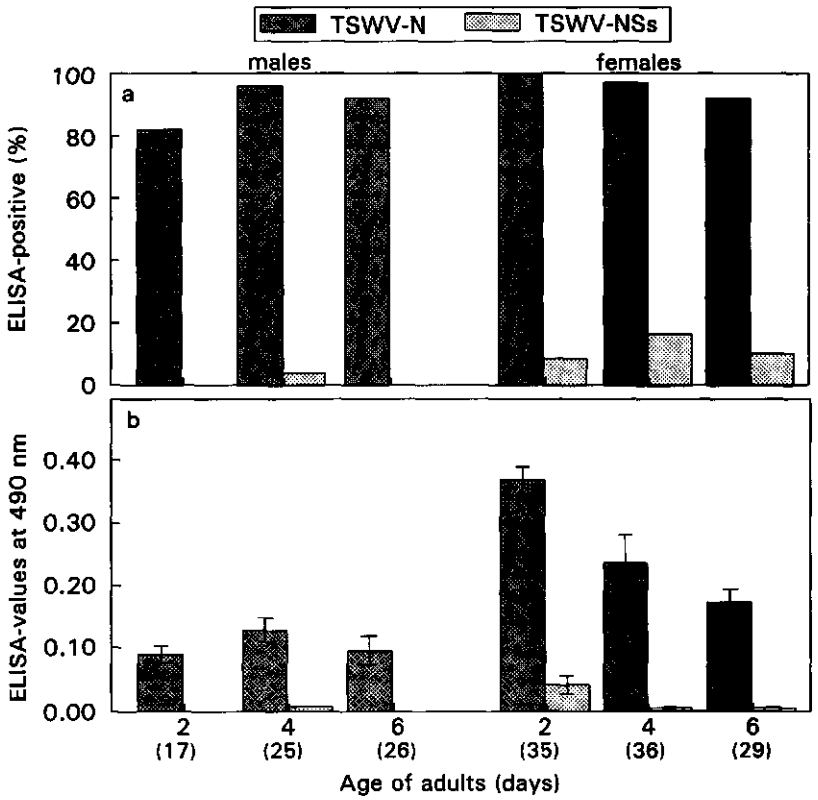

Figure 4. Food consumption by ageing adult male and female *F. occidentalis*, as determined by tomato spotted wilt tospovirus (TSWV) ingestion using enzyme-linked immunosorbent assay (ELISA). Antisera against the TSWV-nucleocapsid protein (N) and a TSWV-nonstructural protein (NS_s) were used. The percentage ELISA-positive adults (a) and average (± s.e.) ELISA values at 490 nm (b) were determined. All readings above the average value of noninfected thrips + 3 times s.d. were considered ELISA-positive. Number of thrips is indicated in parentheses.

The NS_s-protein was detected only in a limited number of thrips (4% of males and up to 17% of females). It is generally accepted that tospovirus ingestion by adult thrips does not result in replication and subsequent transmission (Ullman *et al.*, 1992). Hence, the presence of the NS_s-protein in thrips after TSWV ingestion by adults is not a result of virus replication in the thrips, but due to the presence of this protein in the ingested, virus-containing plant material.

Table 2. Tomato spotted wilt tospovirus (TSWV) transmission by adult males and females from different *Frankliniella occidentalis* populations after three successive inoculation access periods of two days.

	Male		Female		
Population	n[1]	Transmission	n[1]	Transmission	
US2	11	36%	32	13%	**[2]
NZ	30	70%	60	18%	***
US1	11	45%	56	20%	*
NL5	12	50%	23	22%	*
FR	11	64%	21	29%	**
NL1	8	75%	58	31%	*
NL2	11	55%	24	33%	
IS1	9	56%	18	33%	
NL3	26	61%	44	41%	*
JA	15	47%	56	45%	
NL4	21	81%	21	48%	
DK	23	65%	33	58%	
IT	24	79%	41	63%	
IS2	14	79%	34	74%	

[1] Number of thrips tested; [2] Asterisks indicate significant differences between males and females, * $P<0.05$, ** $P<0.01$, *** $P<0.001$ according to Chi-squared statistics.

TSWV transmission by males and females of different F. occidentalis *populations*

To investigate whether the high virus transmission efficiency of adult males is a general phenomenon, fourteen *F. occidentalis* populations, which originated from different locations all over the world, were compared (Table 2). The males of all populations transmitted the virus with a higher efficiency than females. The differences between males and females

were significant when females transmitted TSWV at low efficiencies, as observed for the populations FR (France), NL1, NL2 and NL5 (the Netherlands), NZ (New Zealand), and US1 and US2 (USA). The three populations IS2, NL3 and US2, which were the most efficient, a moderate, and the poorest transmitters (see also Chapter 3), respectively, were selected to quantify their TSWV-N protein content (ELISA) and to correlate this with their transmission competencies. Three groups were distinguished in each population; thrips that were ELISA-positive and able to transmit TSWV, those that were positive in ELISA but failed to transmit, and thrips that were negative in ELISA and nontransmitters (Fig. 5).

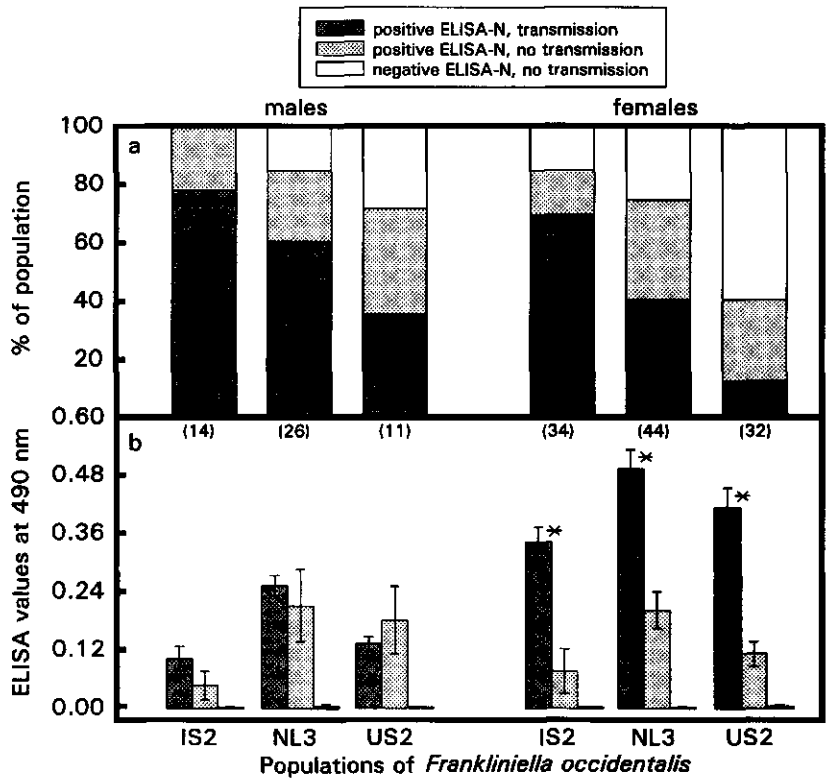

Figure 5. Enzyme-linked immunosorbent assay (ELISA) against nucleocapsid protein of tomato spotted wilt tospovirus (TSWV) results combined with TSWV transmission to petunia leaf disks after three successive inoculation access periods of 2 days. Adult males and females of *Frankliniella occidentalis* populations from Israel (IS2), the Netherlands (NL3) and USA (US2) were used. Numbers of thrips used are indicated in parentheses. The percentage of (non) transmitting thrips and/or ELISA-positive (a) as well as the average ELISA-values (± s.e.) (b) were determined. All values above the average value of noninfected thrips + 3 times s.d. were considered ELISA-positive. Asterisks indicate significant differences between ELISA-positive transmitters and nontransmitters as analysed with Chi-squared statistics at $P<0.005$.

The percentage of thrips that were ELISA-positive was higher than the percentage that transmitted virus to petunia leaf disks. The IS2 population consisted of the highest percentages of ELISA-positive males and females, whereas both sexes of the US2 population transmitted at the lowest efficiencies (Fig. 5a). These results confirm earlier observations that the competency to transmit is related to the virus content (Chapter 2 and 5). Virus content of viruliferous and nonviruliferous males, of all three populations tested, did not differ significantly (Fig. 5b). However, significant differences between transmitting and nontransmitting ELISA-positive females were found for the three thrips populations, those able to transmit contained more viral antigen than those that were not viruliferous.

Discussion

The results presented in this study demonstrate that adult males and females of *F. occidentalis* of the Dutch population NL3 have different virus vector competencies, i.e. 57% of the males transmitted TSWV when only 32% when females transmitted the virus (Fig. 1). As also the males of the thirteen other populations tested exhibited higher transmission efficiencies than females (Table 2), it is suggested that the high TSWV vector competency by males is a general phenomenon for *F. occidentalis*.

Successful TSWV transmission is characterised by injecting virus-containing saliva into a viable cell which can support virus replication and, hence, forward TSWV infection. Differences in virus transmission efficiencies between male and female *F. occidentalis* are likely caused by a distinct feeding behaviour between both sexes. Females often feed intensively, destroying cells in such a way that they fail to support virus replication (Chapter 5). This might explain that although a higher TSWV content is present in females, a lower level of enhanced TSWV infection is found (Fig. 5b). In addition, the high TSWV transmission competency by males is probably the result of their high mobility and probing activity by puncturing many cells, often leaving them suitable for virus replication (Fig. 3a; Chapter 5). In this way, the chance that (virus-containing) saliva is injected in viable cells is that high, that relatively small amount of virus are sufficient to forward transmission (Fig. 5b).

Young adult thrips (both males and females) are more efficient in transmitting TSWV than elder thrips (Fig. 3b), a phenomenon which corresponds with a higher food ingestion (Fig. 4b). As thrips feed more intense in the days directly after adult emergence, probably due to the need for extra energy to recover from the nonfeeding pupal stages and the start of egg production (only females), excretion of virus-containing saliva and/or feeding on a higher number of cells is favoured and so is virus transmission. Another effect of thrips age on virus transmission was that tospovirus transmission proved to be a function of virus content in the thrips. The maximal TSWV transmission, expressed as the percentage

viruliferous adults of the population, was reached when the virus titer (TSWV-N) in thrips was at maximal level at four days after emergence, after which the virus replication was decreased to a very low rate (Figs. 1, 2a and b).

As major differences in TSWV transmission efficiencies between populations and thrips ages now appear to exist, it may be necessary to assess viruliferous adults in the field several times during the cropping season to accurately evaluate TSWV spread in the crop. ELISA, using antiserum against TSWV-N, followed by enzyme amplification represents a reliable method for monitoring viruliferous adults. It was demonstrated that ELISA-N results matched the transmission results, as obtained with the petunia leaf disk assay (Fig. 5a). In this study we could not discriminate between ingested or replicated virus in adults, based on ELISA-values (Figs. 2b and 4b). For monitoring viruliferous thrips in the field, it is essential to put thrips first on non-tospovirus-infected material, to clear the insects from ingested virus prior to ELISA (Cho *et al.*, 1989).

The number of the viruliferous thrips in a population, the sex ratio, and life-history parameters like development time and adult lifetime will determine the efficiency by which tospovirus is transmitted in the field. It was shown (Table 1) that the development time is similar for males and females and that females live twice as long as males, conform previous studies at 25°C (Gerin *et al.*, 1994; van Rijn *et al.*, 1995). The higher transmission efficiency by adult males might neutralise the male's shorter life span, resulting in similar contributions of males and females to TSWV spread in a crop. On the other hand, males might play a major role in the introduction and spread of TSWV in a crop due to their higher mobility (Chapter 5), sex-ratios among flying thrips (Terry and Kelly, 1993) and higher frequency of probing by males (Fig. 3a; Chapter 5). To predict or evaluate spread of TSWV, it is therefore essential to determine the percentage of viruliferous thrips and the sex ratio within a thrips population during the cropping season (Higgins and Myers, 1992). For transmission experiments on laboratory scale, it is crucial to determine tospovirus transmission for both sexes separately, as fluctuations in sex ratio of a population will lead to significant differences in transmission potencies, and therefore to inaccurate conclusions.

Acknowledgements

This research was supported by fellowship ABI22.2704 of the Foundation of Dutch Technology (STW) of the Netherlands Organisation for Scientific Research (NWO). We thank Novartis B. V., Enkhuizen, the Netherlands, for supplying us with *Petunia x hybrida* cv. 'Polo Blauw' seeds. We thank dr. W. J. de Kogel for collecting and maintaining thirteen *F. occidentalis* populations at the Centre for Plant Breeding and Reproduction Research (CPRO-DLO), the Netherlands, and dr. C. Mollema for useful discussions and his continuous interest in our work.

Chapter 5

Distinct feeding behaviour between sexes of *Frankliniella occidentalis* results in higher scar production and lower tospovirus transmission by females*

Abstract

Feeding behaviour and scar production of male and female *Frankliniella occidentalis* were studied in relation to transmission of tomato spotted wilt tospovirus (TSWV). Electrical penetration graph (EPG) analysis showed that females feed more frequently and intensively than males. The feeding intensity, reflected by silvery scar production and studied by an image analysis system, demonstrated that females induced more numerous scars than males. At the same time, males transmitted TSWV with a higher efficiency than females, indicating that TSWV transmission and scar production are not positively correlated. Further, males produced significantly more local lesions of TSWV than females. These quantitative differences in scar production and transmission of TSWV can be explained by the lower mobility and higher consumption rate of females. The influence of the sex ratio on crop damage and virus transmission, and thus to the spread of TSWV, is emphasised.

*This chapter has been published as: van de Wetering, F., Hulshof, J., Posthuma, K., Harrewijn, P., Goldbach, R. and Peters, D. 1998. Distinct feeding behaviour between sexes of *Frankliniella occidentalis* results in higher scar production and lower tospovirus transmission by females. Entomologia Experimentalis et Applicata 88: 9-15.

Introduction

Tomato spotted wilt tospovirus (TSWV) causes severe losses in a wide range of vegetable and ornamental plant species in tropical, subtropical and temperate climate zones (Goldbach and Peters, 1994). One of the main factors contributing to the world-wide occurrence of TSWV is the global dispersal of its vectors: the thrips (Thysanoptera: Thripidae). TSWV is successfully transmitted only after its acquisition by young larvae (Chapter 2), its replication and circulation in the thrips, and its release into saliva (Ullman *et al.*, 1993; Wijkamp *et al.*, 1993). Of the reported thrips species transmitting TSWV, *Frankliniella occidentalis* (Pergande) is evidently one of the most efficient vectors (Wijkamp *et al.*, 1995). This species, whose range expanded almost world-wide in the past decade, is a serious pest in economically important agricultural and horticultural crops (Anonymous, 1993). It is known to be a highly polyphagous insect, feeding on at least 244 species within 62 families (Anonymous, 1989). Thrips not only cause indirect damage by transmitting TSWV, but also produce direct feeding damage, such as scarring, distortion and silvering of infested plant parts. The effects of these insects and the virus results in a severe reduction of aesthetic quality and loss of production (Lewis, 1973).

Frankliniella occidentalis, like all other thrips species, is a piercing-sucking feeder (Hunter and Ullman, 1989). The feeding or foraging process includes intervals of probing or stylet penetration, and of non-probing. When not probing, thrips roam, search for new feeding sites or scrape their heads. When probing, the insects alternate salivation with partial or complete ingestion of cell contents (Chisholm and Lewis, 1984; Harrewijn *et al.*, 1996). Based on the resulting damage, the feeding behaviour of thrips can be classified into two types: penetration and shallow feeding (Sakimura, 1962). Penetration feeding is characterised by feeding on mesophyll cells, resulting in an extreme plasmolysis or complete disappearance of cells. Air then replaces the void cells, causing a silvery, scarred appearance to the affected tissue. Shallow feeding is mainly restricted to epidermal tissues or to a few adjacent mesophyll cells. Here, thrips make a number of feeding probes, which result in tiny silvery patches, but without obvious scarring of the plant (Chisholm and Lewis, 1984).

The frequency and duration with which thrips engage in either penetration or shallow feeding, may differ for males and females, due to behavioural and size differences between the sexes (Tommasini and Maini, 1995). Hence, females (larger than males) might consume more and therefore contribute to higher levels of silvery scar production. If so, scar production is not only the result of the number of thrips, but also depend on the sex ratio within a population. Different feeding behaviour of males and females also might affect the efficiency with which thrips transmit virus. If differences in virus transmission between the sexes of thrips exist, the sex ratio of thrips populations would be crucial to the prediction and evaluation of TSWV spread.

In this study, the feeding behaviour of male and female *F. occidentalis* was monitored using the electrical penetration graph (EPG) and an image analysis system. The frequency and duration of distinct feeding activities of both sexes were correlated with TSWV transmission. Transmission of virus by both sexes of thrips was then investigated in more detail by the analysis of virus symptom induction on petunia.

Materials and methods

Thrips and virus source

Virus-free colonies of *F. occidentalis* were reared on bean pods (*Phaseolus vulgaris* L. cv. 'Prelude'), supplemented with a 10 % sucrose solution and some mm^3 of a commercial pollen mixture at 27 ± 0.5°C with 16/8 h of light/dark. The thrips culture was originally started with adults that were collected from an infestation of greenhouse beans, in the Netherlands. For the transmission studies, the Brazilian isolate (BR-01) of TSWV was used (de Ávila *et al.*, 1990). This isolate was maintained by inoculation of *Datura stramonium* L. plants with viruliferous *F. occidentalis. D. stramonium* plants used for acquisition feeding were mechanically inoculated on the first two true leaves with extracts from thrips-inoculated plants. The infected and noninfected plants were grown in a greenhouse at approx. 22°C (16/8 h of light/dark).

Rearing of viruliferous thrips

Larvae of *F. occidentalis,* up to 4-h-old, were confined on TSWV-infected leaves in Tashiro cages (Tashiro, 1967). All systemically infected leaves had similar virus titers, as determined in enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977; Resende *et al.*, 1991). These leaves were cut into four to six pieces, which were randomly divided over the cages. After an acquisition access period (AAP) of 24 h, the larvae were transferred to noninfected *D. stramonium* leaves in Tashiro cages and reared until the adult stage. The sex of the adult thrips was then determined. The ability of each sex to transmit TSWV was assessed by confining the thrips individually on leaf disks of *Petunia x hybrida* cv. 'Polo Blauw' in two inoculation access periods (IAPs) of 48 h (Wijkamp and Peters, 1993). After each IAP, the petunia leaf disks (13 mm diameter) were incubated on water for three days to develop symptoms, which appear as black to brown local lesions. The transmission efficiency was calculated as the number of viruliferous thrips divided by the total of thrips tested. All experiments were carried out at 25 ± 0.5°C.

Electrical recording of feeding behaviour

The EPG technique (DC method; Harrewijn *et al.*, 1996) was used to monitor thrips feeding behaviour. One electrode, consisting of a 10 μm gold wire, was fixed on the dorsal side of the insect's thorax with a drop of silver paint and connected to the amplifier. The other electrode was put in the soil of a petunia plant. The EPG equipment was placed in a climatised room, which was isolated from electrical noise. After starving the thrips for approximately 1 h, the individual feeding behaviour of ten viruliferous males and females was recorded for 8 h on leaves of petunia plants. As a control, the behaviour of non-viruliferous thrips was also monitored. All thrips used were 7- to 8-day-old adults. A high frequency recorder (Graphtex WTR 771A) printed the results on paper, permitting comparison of the recordings. The petunia leaf on which the thrips had fed, was removed from the plant after 8 h and placed on water for three days for symptom development.

The distinct EPG feeding patterns: extensive feeding, probing with and probing without sap intake, were correlated with TSWV transmission by testing viruliferous females individually on leaf disks of petunia. After an EPG feeding pattern, or a combination of patterns, the feeding site was cut from the leaf and incubated at 27 $\pm$0.5 °C for three days for symptom development.

Relation between scar production and TSWV transmission

These experiments were started with 0- to 4-h-old larvae. The ability of thrips to transmit TSWV was tested in two IAPs of 48 h, directly after adult emergence. These two periods were selected as the maximal virus transmission efficiency is generally reached in the second IAP (Wijkamp *et al.*, 1996b). After each IAP, the area damaged by an individual thrips was monitored using an image analysis system. This system consists of a high-resolution CCD video camera, a colour monitor, a computer, and a frame-grabber (Mollema *et al.*, 1992). Only the upper side of the leaf disks was monitored, as thrips prefer to feed on this side of the petunia leaf. Subsequently, the petunia leaf disks were incubated on water for symptom development. At the end of each experiment, TSWV transmission efficiency, percentage of thrips which produced scars, leaf area damaged by scars, and scar production by viruliferous thrips were separately calculated for males and females. This experiment was performed three times, using a total of 213 females and 92 males in the first, and 182 females and 63 males in the second IAP of 48 h.

Local lesion production on petunia

The number of local lesions on the petunia leaf disks infected by viruliferous thrips was counted and the area covered by these lesions monitored. For analysing the results, the disks were divided into five categories, based on the number of local lesions: 1-3, 4-6, 7-9, >9, NC (not counted, as the lesions were overlapping) and in four categories, based on

the area covered with local lesions: 0-25%, 25-50%, 50-75%, and 75-100%. The disks were then frozen at −20°C prior to ELISA. Leaf disks from noninfected petunia were used as controls in ELISA. The average ELISA-positive value, read at 405 nm, was calculated for each category and sex. Disks with values above the threshold value (avg. control + three times s.d.) were denoted ELISA-positive. This experiment included 115 infected disks inoculated by 73 females, and 101 infected disks inoculated by 58 males.

Results

Electrical recording of feeding behaviour

Clear differences were observed between the feeding behaviour of males and females (Fig. 1), irrespective of whether viruliferous or nonviruliferous thrips were used. When inspected visually, it was evident that females fed more frequently and for longer intervals, while males fed occasionally coinciding with a higher mobility. Attachment of antenna and exposure to a small current did not prevent the thrips from transmitting TSWV during this 8-h period of monitoring. To correlate virus transmission with distinct EPG feeding patterns (Harrewijn *et al.*, 1996), shorter inoculation periods were used. Usually, the starved thrips walked for five to ten min (non probing). Most of them then started probing the leaf for one to 15 s. Approximately 30% of the thrips started extensive feeding directly after walking. This feeding sometimes exceeded 1 h. In some cases, penetration of a cell was only followed by salivation, without sap ingestion. However, no petunia disks were infected with TSWV during these periods, indicating that no correlation could be made between an EPG pattern (or a combination of patterns), and TSWV transmission.

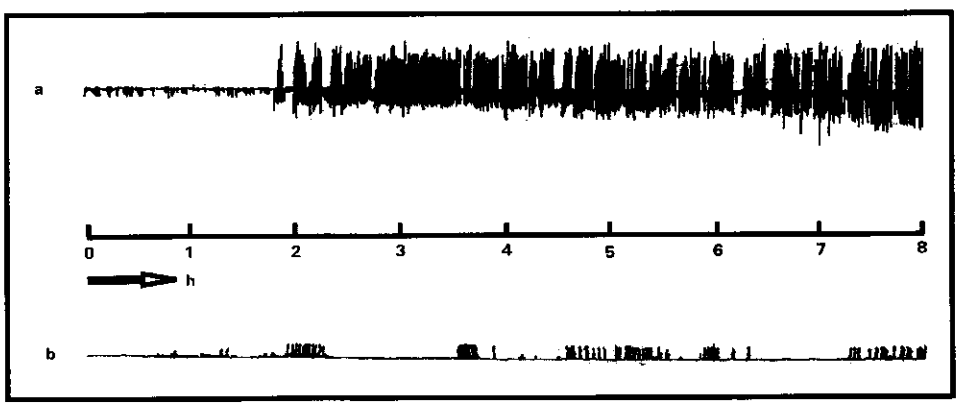

Figure 1. EPG recordings of *Frankliniella occidentalis* for 8 h on petunia: a) female, and b) male thrips. The peaks represent feeding.

Relation between scar production and TSWV transmission

The feeding behaviour of males and females was studied in relation to (silvery) scar production and TSWV transmission. In this study, in which the local lesion host petunia was used, silvery scars induced by feeding injury could easily be distinguished from the black to brown local lesions caused by TSWV. The percentage of females, which produced scars, was notably higher than that of males (Fig. 2). As expected, females damaged a larger leaf area than males (Table 1), although this difference was not significant when analysed with the Student-*t*-test at $P<0.05$. However, the TSWV transmission efficiency was significantly higher for males than for females (Fig. 3).

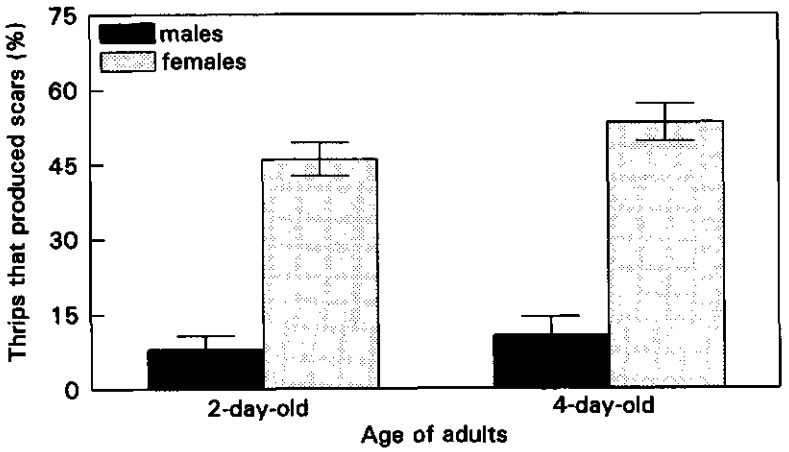

Figure 2. The percentage of *Frankliniella occidentalis* males and females producing scars in two feeding periods of 48 h after adult emergence. Vertical bars indicate s.e.'s.

Table 1. Leaf area damaged (average ± s.e.) by *Frankliniella occidentalis* males and females in two successive feeding periods of 48 h.

	2-day-old adults		4-day-old adults	
	n[1]	damaged area (mm²)[2]	n[1]	Damaged area (mm²)[2]
Male	92	0.60 ± 0.12[a]	63	0.99 ± 0.18[a]
Female	213	0.98 ± 0.08[a]	182	1.29 ± 0.14[a]

[1] Number of thrips tested; [2] Within a column, averages followed by the same letter are not significantly different at $P<0.05$ by Student-*t*-test.

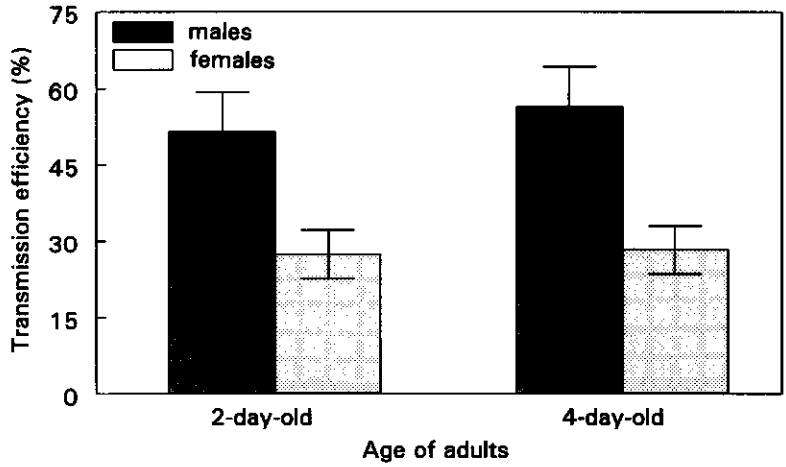

Figure 3. Tomato spotted wilt tospovirus (TSWV) transmission efficiency by males and females of *Frankliniella occidentalis* during two feeding periods of 48 h after adult emergence. Vertical bars indicate s.e.'s.

To determine whether TSWV transmission is related to the production of silvery scars, the percent of viruliferous thrips that caused scars was compared to the percent of those that did not. Viruliferous thrips, which did not cause scar damage, transmitted TSWV more efficiently than those producing scars (Table 2). Moreover, the percentage of viruliferous thrips that caused scars is much lower for males (6 to 15%) than for females (33 to 50%), suggesting that males and females differ in their feeding behaviour.

Table 2. The ability of viruliferous *F. occidentalis* males and females to produce scar damage after two successive feeding periods of 48 h.

	2-day-old adults			4-day-old adults		
	n^1	damage (%)	no damage (%)	n^1	damage (%)	no damage (%)
Male	92	6	94	63	15	85
Female	213	33	67	182	50	50

[1] Number of thrips tested.

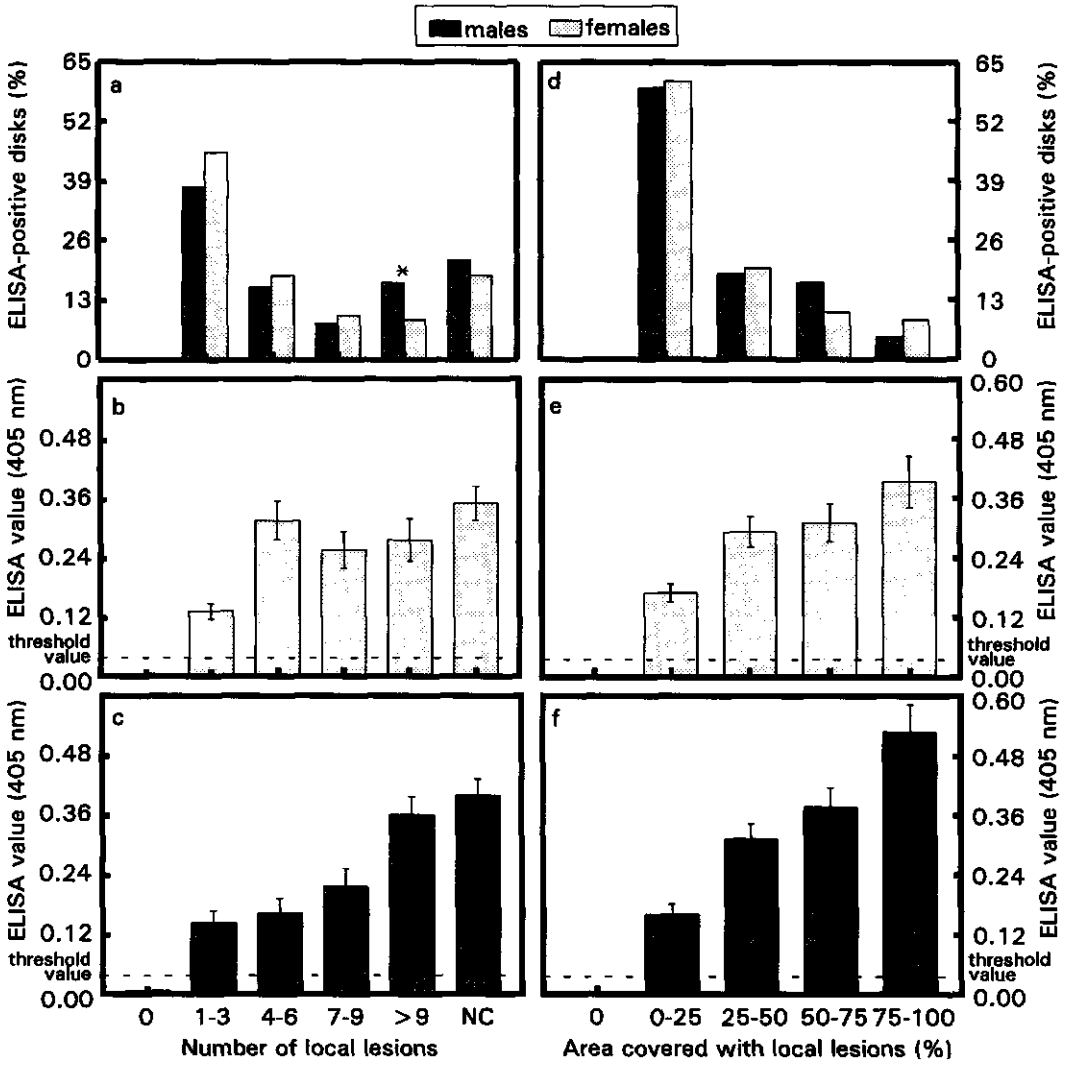

Figure 4. Local lesion production and tomato spotted wilt tospovirus (TSWV) level in petunia leaf disks after inoculation by either *Frankliniella occidentalis* males or females. Percentage of disks showing different number of lesions (a) and the leaf area covered with lesions (d) was estimated. The amount of TSWV viral antigen per ELISA-positive disk, presented as average ELISA value (± s.e.), after inoculation by females (b, e) or males (c, f) is correlated with the number of local lesions and leaf area covered with symptoms, respectively. ELISA values higher than the threshold value (avg. control plus 3 times s.d.) were considered positive. NC = not counted, as the lesions were overlapping. Asterisks indicate significant difference between males and females ($P<0.05$, Chi-squared statistics).

Local lesion production on petunia

To further study virus transmission by males and females, TSWV infected leaf disks were analysed for the number and size of local lesions (Figs. 4a and d). ELISA revealed that all disks with lesions were infected, whereas disks without lesions were not (Figs. 4b, c, e and f). This indicated that the petunia leaf disk assay is a reliable method to score TSWV infection.

A higher percentage of disks, classified in the local lesion category >9 and NC, was infected by males, whereas a higher percentage was found in the categories 1-3, 4-6 or 6-9 when infected by females. A significant difference in the percentage of disks infected by males and females was found in the category >9 (Fig. 4a). These observations show that males make more effective virus inoculation punctures than females. After inoculation by females, the amount of virus in disks reached a maximum level when four or more local lesions were produced (Fig. 4b). However, when males inoculated the disks, the amount of virus increased with the number of local lesions (Fig. 4c). These results show that not always a clear correlation exists between the number of local lesions and the TSWV titer in petunia disks, confirming earlier studies (Wijkamp and Peters, 1993).

The leaf area covered by lesions, as presented in Figure 4d, was similar for males and females. The virus titers in the petunia disks increased with the area covered with local lesions (Figs. 4e and f), and were slightly higher for disks infected by males than for those infected by females. In the future, the leaf area covered with local lesions can be used to estimate the TSWV titer in petunia leaf disks.

Discussion

This study demonstrated that feeding behaviour of male *F. occidentalis* differs from that of females, which results in different levels of scar production and TSWV transmission. Scar production was not positively correlated with TSWV transmission on petunia. This is consistent with previous studies on chrysanthemum (Allen *et al.*, 1990 and 1991; Broadbent *et al.*, 1990 and 1995). TSWV infection is more likely to be caused by short feeding activities like probing or when a small amount of food is ingested. This conclusion is based on the observations that males transmit TSWV with a higher efficiency, that males make more feeding or effective inoculation punctures than females, and that 85 to 94% of viruliferous males do not induce scarring. Since little or no cell content is ingestion by males, the cells are not irreversibly damaged and still able to support viral infection.

The ability of males and females to transmit plant viruses has been studied by a few authors. No sexual differences were reported in the transmission of maize chlorotic dwarf virus (Rodriguez *et al.*, 1993) and rice waika virus (Hirao and Inoue, 1978 and 1979) by leafhoppers. Maize streak virus is more efficiently transmitted by the female cicadellids than by males (van Rensburg and Giliomee, 1990). However, similar to our results, male aphids of *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) are more efficient transmitters of barley yellow dwarf virus (BYDV) than females (Halbert *et al.*, 1992). Hence, we conclude that the sex-ratio in insect populations does not only affect TSWV transmission, but also plays a role in other vector-plant virus relations.

Virus transmission by aphids and leafhoppers is classified as either stylet-borne or circulative. Tospovirus transmission by thrips is somewhat different from the virus-vector interactions described for circulatively transmitted viruses, e.g. BYDV by *R. padi* (Prado and Tjallingii, 1994). First, thrips feed on epidermal tissue and underneath parenchymous cells instead of phloem cells, which are preferred by most insects that are vectors of circulatively transmitted plant viruses. Thrips thrust the stylets straight through the epidermis and into the next layer of cells, and do not actively switch from intercellular to intracellular stylet penetration. Hence, tospovirus transmission by thrips might, in a way, resemble stylet-borne transmission. Second, thrips have a different stylet anatomy than hemipterans, as they have only one stylet canal, used for both saliva egestion and sap ingestion. Hence, salivation is not simultaneous with fluid intake (Harrewijn *et al.*, 1996). Salivation by thrips is crucial for tospovirus transmission, as injection of virus-containing saliva in a viable cell is a prerequisite for tospovirus infection. Third, the efficiency of tospovirus transmission by thrips differs between males and females, as a result of different feeding behaviour. However, which specific feeding activity of thrips leads to a successful infection of a cell remains to be studied.

The feeding activities of thrips, after stylet insertion, can be classified into three different patterns (P, Q, and R), as analysed by EPG (Harrewijn *et al.*, 1996). The P pattern reflects probing of one second or less, the Q probing pattern lasts at least 1-2 sec, while the R pattern lasts over 10 s. The R pattern often induces silvery scars, while patterns P and Q may indicate shallow feeding. The P pattern reflects the onset of penetration, while the Q pattern likely represents salivation, essential for tospovirus transmission. The concentration of virus in the saliva and the viability of the injected cell will determine the probability by which a puncture results in an infection. The fact that no TSWV transmission was found during short inoculation periods indicates that this probability of infection is rather low per puncture. A higher frequency of Q patterns probably results in a higher chance of successful transmission. This implies that the higher virus transmission ability of males is due to higher frequency of probing.

As a result of this study, it can be concluded that both the efficiency of TSWV transmission and scar production by *F. occidentalis* is influenced by the sex-ratio of the population. It is therefore essential to consider the sex-ratio of natural thrips populations in order to study population dynamics (Higgens and Myers, 1992), predict the consequent crop damage and to evaluate the spread of TSWV.

Acknowledgements

This research was supported by fellowship, No. ABI22.2704, of the Foundation of Dutch Technology (STW) of the Netherlands Organisation for Scientific Research (NWO). We thank Novartis B. V., Enkhuizen, the Netherlands, for supplying us with *Petunia x hybrida* cv. 'Polo Blauw' seeds. We also thank M. van der Hoek and dr. F. R. van Dijken of the Centre for Plant Breeding and Reproduction Research (CPRO-DLO), the Netherlands for their technical assistance, and dr. P. Grostal for reading the manuscript.

Chapter 6

Assessment of susceptibility of different chrysanthemum cultivars for tomato spotted wilt virus*

Abstract

Three methods were compared to assess the susceptibility of vegetatively propagated chrysanthemum for tomato spotted wilt tospovirus (TSWV), i.e. mechanical and thrips-mediated inoculation of whole plants, and a leaf disk assay. As symptom expression was often poor or even absent, TSWV infections and subsequent susceptibility for TSWV were determined by enzyme-linked immunosorbent assay (ELISA). All fifteen chrysanthemum cultivars tested in these studies were susceptible for TSWV, irrespective of their degree in vector-resistance (based on feeding scar damage rates). Thrips-mediated inoculation using different numbers of thrips revealed that 100% infection was obtained when plants were challenged by six thrips/plant, whereas 80% and over 50%, respectively, of the plants became infected when inoculated by a single male or female thrips. However, false negatives were scored due to erratic, cultivar specific and time-dependent virus distribution in the plants even after intensive sampling. Labour-intensive samplings and long incubation periods could be overcome by a readily applicable leaf disk assay. This assay was as reliable as thrips-mediated inoculation of whole plants, and its use is therefore favoured to assess chrysanthemum cultivars for TSWV susceptibility.

*This chapter has been accepted for publication in Plant Pathology as: van de Wetering, F., K. Posthuma, R. Goldbach, and D. Peters. Assessment of susceptibility of different chrysanthemum cultivars for tomato spotted wilt virus.

Introduction

The production of greenhouse chrysanthemum (*Dendranthema grandiflora* Tzvelev), an important ornamental crop in the Netherlands and other countries with a floricultural industry, is threatened by various pathogens, including tomato spotted wilt tospovirus (TSWV) (Verhoeven and Cevat, 1996) and its major vector, the western flower thrips, *Frankliniella occidentalis* (Wijkamp *et al.*, 1995). The damage caused by TSWV is often even more devastating than that caused by thrips feeding (silvery scars and deformation) (Roosjen and Cevat, 1995).

In the Netherlands, TSWV was first reported in 1931 (Noordam, 1943). At that time, leaf infections occurred in a few crops while the damage was limited. Since 1983, *F. occidentalis* is the most common and most difficult-to-control pest insect in the Netherlands (Mantel and van de Vrie, 1988). The predominance of *F. occidentalis* in greenhouses resulted in a dramatic increase of TSWV infections at the end of 1980s. The initial unawareness of both symptomatology and epidemiology of the virus by the growers further increased the impact of infections. Also, symptoms were often ascribed to other pathogens or physical disorders, and ineffective measures were taken for control (Verhoeven and Roenhorst, 1998).

On chrysanthemum, the most common type of TSWV symptoms is the formation of chlorotic spots on top leaves followed by wilting, development of severe necrosis on leaves and stem, stunting and death of young plants (Alexandre *et al.*, 1996; Dal Bò *et al.*, 1995; Daughtrey *et al.*, 1997; Matteoni and Allen, 1989). Although chrysanthemum plants can be severely affected by TSWV, symptoms on the infected plants may often develop slowly and remain restricted to a few leaves and/or parts of the stem. Regularly, TSWV infected chrysanthemum even remain symptomless (Matteoni and Allen, 1989).

To date, the impact of TSWV is significantly reduced as a result of improved information and well-directed advises (Verhoeven and Roenhorst, 1998). However, complete control of TSWV has never been established. The current control recommendations for tospoviruses are to rogue infected plants and to control thrips populations by e.g. the use of clean-stock material (Ochoa *et al.*, 1996b), clean production areas (Ochoa *et al.*, 1996b), screens (Robb and Parella, 1989), the use of thrips resistant cultivars (van Dijken *et al.*, 1994; de Jager *et al.*, 1995), and applications of chemicals (Robb and Parella, 1989). Insecticides are still repeatedly used although their use is declining because of e.g. consumers' demands (de Ponti, 1982), and the increased resistance of *F. occidentalis* to pesticides (Brødsgaard, 1994b; Robb *et al.*, 1995; Zhao *et al.*, 1995). Further measures are needed to prevent TSWV spread by e.g. the use of virus- and/or vector-resistant cultivars. However, no chrysanthemum cultivars with complete resistance to TSWV or thrips have been identified so far. Hence, chrysanthemum cultivars are classified as either susceptible, intermediate or (partially) resistant to thrips (based on

the feeding scar damage rates) and TSWV (Allen *et al.*, 1990 and 1991; Broadbent *et al.*, 1990 and 1995; van Dijken *et al.*, 1994; de Jager *et al.*, 1995).

In this study, different chrysanthemum cultivars, which differ in their degree of vector resistance, were tested for their susceptibility for TSWV using the two conventional methods, mechanical and thrips-mediated inoculation of cuttings, and a more rapid and less labour-intensive leaf disk assay (Wijkamp *et al.*, 1996a; Bergh and Le Blanc, 1997).

Materials and methods

Virus isolate and thrips culture

The TSWV isolate BR-01 (de Ávila *et al.*, 1990) was maintained by thrips-mediated passages on *Datura stramonium* L. plants. The *F. occidentalis* population NL3 (Chapter 2), which originated from an infestation on bean in the Netherlands, was used in all experiments. Virus-free colonies of this thrips were reared on *Phaseolus vulgaris* L. cv. 'Prelude' pods at 25 ± 0.5°C supplied with commercially available pollen and a little cup containing 10% sucrose solution and a daily photoperiod of 16 h.

Determination of TSWV infection by enzyme-linked immunosorbent assay (ELISA)

Polyclonal antiserum raised against the nucleocapsid (N) protein of TSWV isolate BR-01 and conjugate were used in a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) format with some alterations (Chapter 2). TSWV infection was monitored in all plants, including mock-inoculated plants, by sampling three inoculated, middle, and top layer leaves. Extracts were prepared by grinding leaf tissue at a 1:50 ratio (w:v). Plants were denoted positive when the ELISA readings were higher than the average value of noninfected material + 3 times s.d.

TSWV infection of chrysanthemum plants upon mechanical inoculation

Three to four weeks old chrysanthemum cuttings were inoculated with extracts prepared from the first true systemically infected leaves of thrips-inoculated *D. stramonium* plants by triturating 1 g of leaf in 5 ml inoculation buffer consisting of 0.008 M NaH_2PO_4, 0.008 M Na_2HPO_4, 0.1 M Na_2SO_3, pH 7,0, at 4°C, or mock-inoculated as controls. The plants were grown in a greenhouse at approximately 20°C at a long day period (16L:8D), regularly inspected for symptom expression and analysed for the presence of viral antigen in DAS-ELISA at six and twelve weeks post inoculation (p.i.). In this experiment, up to 44 plants per cultivar were analysed.

A minimum of six plants of each chrysanthemum cv. 'Statesman', 'Majoor Bosshardt' and 'Sunny Cassa' were used, which were partially resistant, intermediate resistant, or fully susceptible to thrips based on feeding scar damage rates, respectively

(van Dijken *et al.*, 1994). These plants were analysed for infection by ELISA every two weeks. Differences between the percentage of TSWV infected plants between cultivars were analysed using Chi-squared statistics at $P<0.05$ (Rijpkema, 1993; Schulman, 1992).

Thrips-mediated TSWV infection of chrysanthemum plants and leaf disks

Newly hatched larvae of *F. occidentalis*, up to 4 h old, were placed on infected *D. stramonium* leaves for 24 h, and reared on noninfected leaves of this plant species until adult emergence at 25°C. In the adult stage, thrips were individually tested for their ability to transmit TSWV to petunia leaf disks in two inoculation access periods (IAPs) of 48 h (Wijkamp and Peters, 1993). After each IAP, the disks were incubated for three days on water for the development of black to brown local lesions. Viruliferous thrips that were five- to six-day old, were selected and used in the following experiments.

Chrysanthemum cuttings of three to four weeks old were individually inoculated by three, six, nine viruliferous thrips (in a sex ratio of one male to two females), or by a single male or female in a 48-h IAP. After this period, thrips were removed and the plants were monitored for TSWV symptom expression and TSWV infection every two weeks. On average six plants of the cv. 'Statesman', 'Majoor Bosshardt' or 'Sunny Cassa' were used per experiment. The competence of a single male or female to transmit TSWV was tested inoculating respectively five and nine plants of both 'Statesman' and 'Sunny Cassa' plants. The plants were grown in a greenhouse at approximately 20°C with a photoperiod of 16 h. The same number of nonviruliferous thrips challenged control plants.

Leaf disks were thrips-inoculated at the same time as the plants to concurrently optimise the inoculation methods used. Viruliferous thrips were individually placed for 48 h IAP on disks cut from the middle leaves of 'Statesman' ($n=32$) and 'Sunny Cassa' ($n=44$) plants. The disks were incubated at 25 (± 0.5)°C for four days on water for the development of TSWV infection, and analysed by DAS-ELISA. In each test, several control plants or disks were exposed to the same number of nonviruliferous thrips. The percentage of infected (ELISA-positive) disks was compared with the ELISA-positive plants, when challenged by either three or six thrips or by mechanical inoculation, using Chi-squared statistics at $P<0.05$ (Rijpkema, 1993; Schulman, 1992).

Results

TSWV infection of chrysanthemum plants upon mechanical inoculation

Fifteen chrysanthemum cultivars, which differ in vector resistance based on feeding scar damage rates, were tested for their susceptibility for TSWV.

Table 1. Tomato spotted wilt tospovirus (TSWV) infections in mechanically inoculated plants of several chrysanthemum cultivars, which differ in thrips resistance based on the formation of silvery scars on leaves.

Cultivar	No. plants observed	No. weeks p.i.	% ELISA positive plants	Symptom expression %	Symptom expression Type[a]	Thrips resistance[b]
Sunny Cassa	44	6	80	0	CV, S	susceptible
		12	93	20		
Pink Pompon	30	6	70	0	CV, CS	susceptible
		12	100	17		
D. pacifica	7	6	29	0	CS,NL	susceptible
		12	86	43		
Byoux	13	6	23	0	CL, NL	intermediate
		12	77	23		
Reagan	13	6	100	0		intermediate
		12	100	0		
Penny Lane	13	6	100	0		intermediate
		12	92	0		
Daymark	13	6	77	0		intermediate
		12	62	0		
Reyellow	8	6	25	0		intermediate
		12	100	0		
Taube	13	6	100	0	CL	intermediate
		12	31	15		
Majoor	40	6	60	43	CL, NL,	intermediate
Bosshardt		12	68	68	S	
Reaction	13	6	0	0	S	partially
		12	69	69		resistant
Statesman	28	6	54	0		partially
		12	75	0		resistant
Royal Accent	13	6	77	0		partially
		12	100	0		resistant
Ellen van	26	6	92	23	CL, NL,	not known
Langen		12	96	12	S	
Peach Fiji	36	6	92	14	CS, CV	not known
		12	83	22		

[a] NL: necrotic lesions, CL: chlorotic lesions, CS: chlorotic spots, CV: chlorotic veins, S: stunting; [b] According to Van Dijken *et al.* (1994).

As only a limited number of plants of some cultivars tested showed clearly visible symptoms, varying between chlorotic veins, chlorotic spots and lesions, stunting and necrotic lesions (Table 1), ELISA was used to determine TSWV infections in the plants, and subsequent virus susceptibility of the cultivars. All cultivars appeared susceptible to TSWV as most, up to 100% (see Table 1), of the plants became infected (ELISA-positive) regardless of their degree of vector-resistance. For most cultivars, maximal TSWV infection levels, i.e. the highest percentage of ELISA-positive plants, were found at twelve weeks p.i.,. However, for the cv. 'Daymark', 'Peach Fiji', 'Penny Lane', 'Reagan', and 'Taube' this point was already reached at six weeks p.i. As for most of these cultivars the percentage of plants found to be infected was lower when sampled 12 weeks p.i., it is suggested that some infected plants were scored as false negatives even after our intensive sampling procedure.

A few cultivars were selected to follow the development of TSWV infection after mechanical inoculation more precisely (Fig. 1). Maximal percentages of infected plants were obtained six to eight weeks after inoculation, of which a slightly higher percentage (83%) of ELISA-positive plants were found for 'Sunny Cassa' (fully susceptible to thrips), than for the other two cultivars tested, 'Statesman' and 'Majoor Bosshardt' (partially resistant and intermediate resistant to thrips, respectively), of which 67% of the plants became infected (Fig. 1). This difference was not significant as tested by Chi-squared statistics ($P<0.05$).

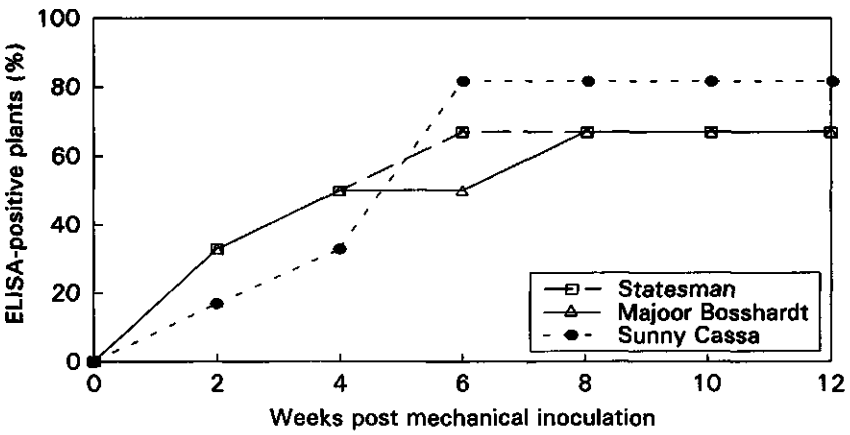

Figure 1. The percentage enzyme-linked immunosorbent assay (ELISA)-positive plants in time, after mechanical inoculation. A partially resistant chrysanthemum cv. 'Statesman', an intermediate resistant cv. 'Majoor Bosshardt', and a thrips fully susceptible one 'Sunny Cassa', based on the feeding scar damage rates on leaves, were used. Plants were denoted positive when the ELISA readings were higher than the average value of noninfected plant material + 3 times s.d.

Thrips-mediated TSWV inoculation of chrysanthemum plants

Besides mechanical inoculation, thrips-mediated inoculation was used to assess the susceptibility of TSWV for a selection of chrysanthemum cultivars, i.e. 'Statesman', 'Majoor Bosshardt', and 'Sunny Cassa', as this method reflects the natural inoculation. As symptom expression upon inoculation developed rather slowly (4 weeks), or was even absent (Table 2), ELISA was used again (Table 1) to monitor TSWV infection.

Table 2. Percentage of chrysanthemum plants showing tomato spotted wilt tospovirus symptoms (TSWV) 2 to12 weeks post thrips inoculation. 'Statesman' is a partially resistant, 'Majoor Bosshardt' an intermediate susceptible and 'Sunny Cassa' a fully susceptible cultivar to thrips, based on the formation of silvery scars on leaves.

	% plants with symptoms/weeks p.i.					
Cultivar	2	4	6	8	10	12
Statesman	0	0	0	0	0	0
Majoor Bosshardt[1]	0	6	6	6	6	6
Sunny Cassa[2]	0	14	23	23	23	23

Type of symptoms observed: [1] stunting; [2] chlorosis and necrosis on leaves.

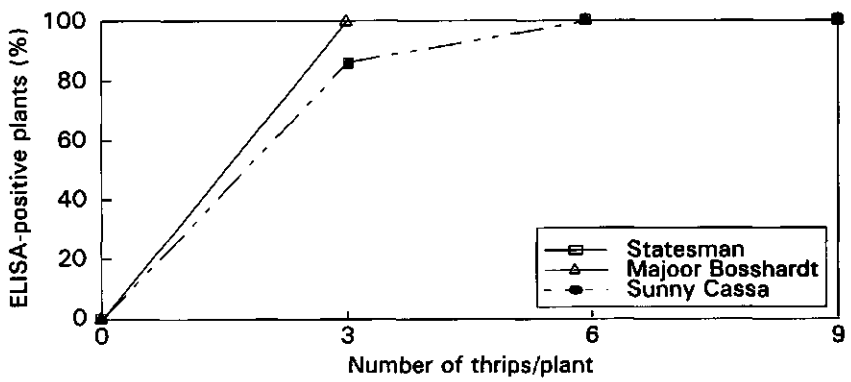

Figure 2. The percentage enzyme-linked immunosorbent assay (ELISA)-positive plants after a 48 h inoculation access period using different numbers of viruliferous thrips. A partially resistant chrysanthemum cv. 'Statesman', an intermediate resistant cv. 'Majoor Bosshardt', and a thrips fully susceptible one 'Sunny Cassa', based on the feeding scar damage rates on leaves, were used. Plants were denoted positive when the ELISA readings were higher than the average value of noninfected plant material + 3 times s.d.

Conform results obtained with mechanical inoculations, all chrysanthemum cultivars tested were fully susceptible for TSWV (Fig. 2). The impact of the number of viruliferous thrips on TSWV infections was large, as single male and female *F. occidentalis* infected 80% and over 50% of the chrysanthemum plants (Table 3) (Chapter 5), whereas maximal infection levels were obtained when three ('Majoor Bosshardt'), six ('Statesman', 'Sunny Cassa') or more viruliferous thrips challenged the plants. The maximal infection levels of 100% infected plants were only obtained after six weeks p.i. for the cv. 'Statesman' and 'Majoor Bosshardt', and four weeks p.i. when the cv. 'Sunny Cassa' was analysed (Fig. 3).

Table 3. Percentage tomato spotted wilt tospovirus (TSWV) infected chrysanthemum plants that were inoculated by either a viruliferous male or female of *F. occidentalis* for 48 h. 'Statesman' is a partially resistant and 'Sunny Cassa' a fully susceptible cultivar to thrips, based on the formation of silvery scars on leaves.

Cultivar	Female	Male
Statesman	56%	80%
Sunny Cassa	78%	80%

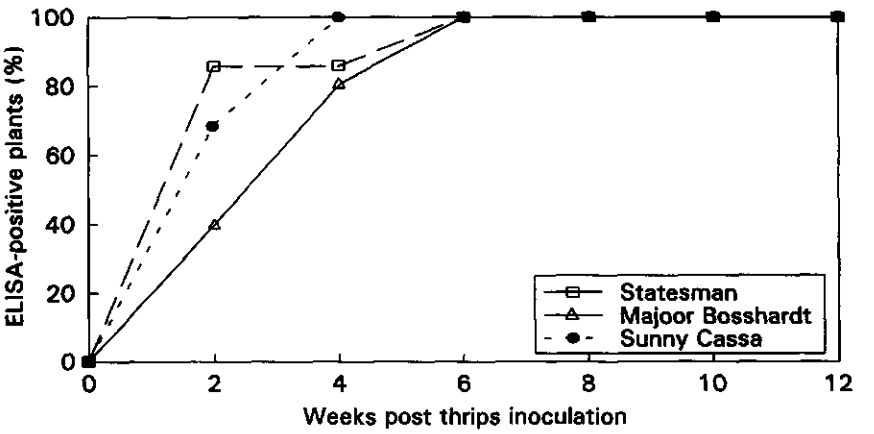

Figure 3. The percentage enzyme-linked immunosorbent assay (ELISA)-positive plants in time, after a 48 h inoculation access period by six viruliferous thrips. A partially resistant chrysanthemum cv. 'Statesman', an intermediate resistant cv. 'Majoor Bosshardt', and a thrips fully susceptible one 'Sunny Cassa', based on the feeding scar damage rates on leaves were used. Plants were denoted positive when the ELISA readings were higher than the average value of noninfected plant material + 3 times s.d.

The distribution of TSWV in plants differed considerably with time (Fig. 4). Depending on the cultivar, the TSWV content in the inoculated leaves reached maximum values between four and eight weeks p.i., after which it decreased probably by virus degradation. TSWV infection in the middle and top leaves developed gradually; shortly after inoculation no TSWV-antigen was detected whereas a considerable amount, above the threshold value, was found 10 to 12 weeks p.i. The distribution within these leaves was cultivar specific, as e.g. the virus content in the middle leaves differed considerably between cultivars compared to the amount of viral antigen in their inoculated leaves. As a result, it can be concluded that the distribution of TSWV within chrysanthemum plants is erratic, cultivar-specific and changes in time and, hence, explains finding of possible false negatives even after frequent and intensive sampling.

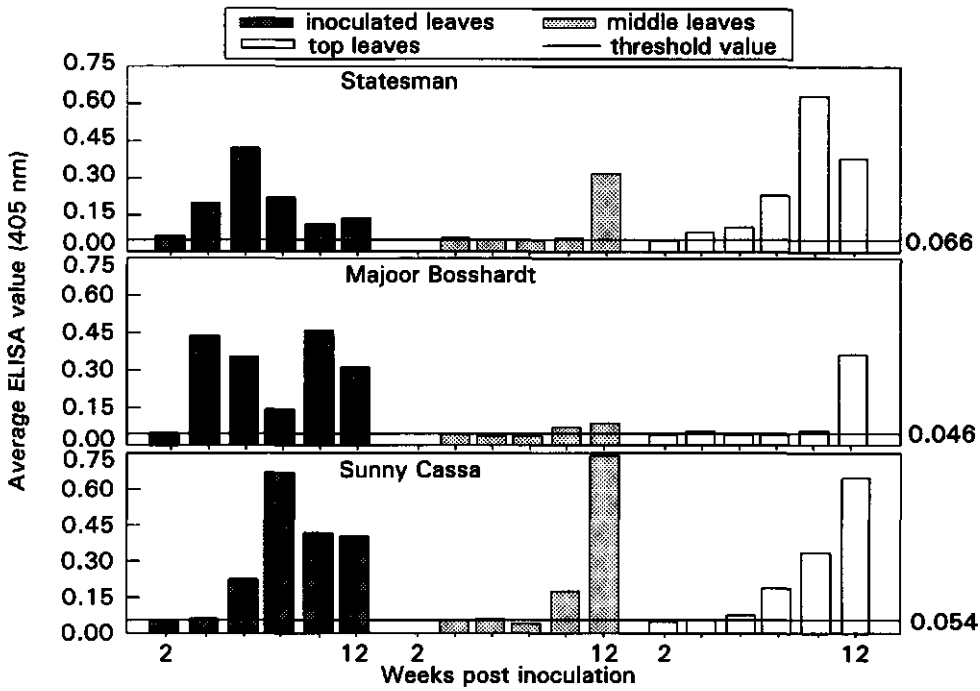

Figure 4. The enzyme-linked immunosorbent assay (ELISA)-readings at 405 nm of the inoculated, top and middle leaves of the infected plants in time. The plants were inoculated with six thrips for 48-h inoculation access period. A partially resistant chrysanthemum cv. 'Statesman', an intermediate resistant cv. 'Majoor Bosshardt', and a thrips fully susceptible one 'Sunny Cassa', based on the feeding scar damage rates on leaves, were used. Plants were denoted positive when the ELISA readings were higher than the threshold value, calculated as the average value of noninfected plant material + 3 times s.d.

Development of a chrysanthemum leaf disk assay

In view of the labour-intensive samplings, false negatives, and long incubation periods of infections when whole plants were inoculated (either mechanically or by thrips), a leaf disk assay was introduced to assess TSWV susceptibility of chrysanthemum. This leaf disk assay is easily executed and only requires six days, in stead of six weeks when plants were inoculated. Slight differences were found in TSWV susceptibility between cv. 'Statesman' (95%) and 'Sunny Cassa' (84%), conform virus inoculations of plants (Fig. 5). The outcomes of this assay differed significantly from the results obtained upon mechanical inoculation, whereas the percentage of infected disks could be well correlated with the percentage ELISA-positive plants obtained upon thrips-mediated inoculation (Chi-squared statistics, $P<0.05$).

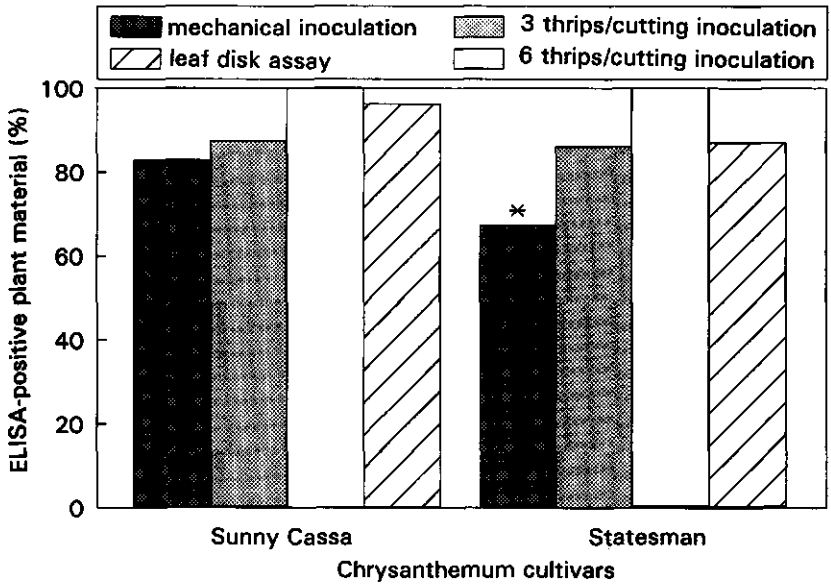

Figure 5. Comparison of leaf disk assay with the inoculation of plants mechanically and by three or six viruliferous thrips, using as parameter the percentage enzyme-linked immunosorbent assay (ELISA)-positive plant material. Chrysanthemum plants were challenged by viruliferous thrips for 48 h. A partially resistant chrysanthemum cv. 'Statesman' and a thrips fully susceptible one 'Sunny Cassa', based on feeding scar damage rates on leaves, were used. Plants were denoted positive when the ELISA readings were higher than the average value of noninfected plant material + 3 times s.d. Asterisk indicate significant difference compared with leaf disk assay ($P<0.05$, Chi-squared statistics).

Discussion

All fifteen cultivars tested in these studies turned out to be fully susceptible to TSWV (Table 1), irrespective of their degree of vector resistance, which is based on feeding scar damage rates. Hence, future research should reveal whether chrysanthemum cultivars can be identified or developed with a high degree of both TSWV and vector resistance. To simplify the assessment of susceptibility of chrysanthemum cultivars for TSWV in this research, a chrysanthemum leaf disk assay was introduced. This assay is executed easily, is less time-consuming (six days in stead of six weeks) and labour-intensive than the two conventional methods, i.e. mechanical and thrips-mediated inoculation of whole plants, and reliably reflects the natural, i.e. thrips-mediated inoculation of chrysanthemum plants (Fig. 5).

The susceptibility of different chrysanthemum cultivars for TSWV is assessed by the percentage of infected chrysanthemum disks or plants. Despite the high levels of TSWV infected plants obtained upon virus inoculation, of up to 100% (Figs. 1 and 3), symptom expression was often poor or even absent in the majority of cultivars tested (Tables 1 and 3). Hence, ELISA was needed to assess TSWV infection in the plant. Conform previous studies (Allen *et al.*, 1990), this assay was employed only in the leaves, as other parts of the plants are needed for consumer demands, or are more easily infected by bacterial or fungal infection. Intensive sampling procedures assaying three leaves of the top, middle and inoculated leaf layers every two weeks, did not avoid false negatives due to an erratic, cultivar specific and time dependent TSWV distribution in plants (Fig. 4). To overcome such unnoticed infections, the leaf disk assay is required to reliably assess TSWV susceptibility, whereas indicator plants such as petunia (Allen and Matteoni, 1991) and the tobacco species *Nicotiana benthamiana* (Daughtrey *et al.*, 1997) should be used to accurately identify TSWV and viruliferous thrips in the cropping area.

To date, TSWV control in chrysanthemum largely depends on vector identification and management by preventing the crop from incoming viruliferous thrips and chemical thrips control. The use of chemicals in chrysanthemum, however, is not completely adequate due to the inaccessible hide-outs of thrips, increasing resistance of *F. occidentalis* to pesticides (Brødsgaard, 1994b; Robb *et al.*, 1995; Zhao *et al.*, 1995), the short inoculation periods before they are killed (Wijkamp *et al.*, 1996c), and the fact that already small numbers of viruliferous thrips can severely infect a chrysanthemum crop (Fig. 2 and Table 2). As chemical control is ineffective, alternative vector management is needed to prevent and limit TSWV spread in the chrysanthemum production area, such as the use of thrips and TSWV resistant cultivars. Currently, some chrysanthemum cultivars with some levels of TSWV resistance have been developed (Cho *et al.*, 1996; Daughtrey *et al*, 1997; Sherman *et al.*, 1996), although it is still unknown if such cultivars adequately reduces TSWV spread by thrips. Hence, future research on TSWV susceptibility on chrysanthemum cultivars

should reveal whether resistant cultivars can be identified and used to increase durability of TSWV management programs.

Acknowledgements

This research was supported by fellowship, No. ABI22.2704, of the Foundation of Dutch Technology (STW) of the Netherlands Organisation for Scientific Research (STW). The authors thank Novartis B. V., Enkhuizen, the Netherlands, for supplying us with *Petunia x hybrida* cv. 'Polo Blauw' seeds. We also gratefully thank Mr. S. van der Knaap of Fides Plants and M. T. A. Dik of the Centre for Plant Breeding and Reproduction Research (CPRO-DLO), the Netherlands for their supply of chrysanthemum plants. The useful discussions and continuous interest in our work of dr. C. Mollema (CPRO-DLO) is gratefully acknowledged.

Chapter 7

Adverse effects of vector resistance on tomato spotted wilt virus infection in chrysanthemum*

Abstract

Tomato spotted wilt virus (TSWV) transmission to the chrysanthemum cv. 'Statesman' and 'Sunny Cassa', which are partially resistant and susceptible to thrips silvery scar production, respectively, was quantified. Using a leaf disk assay, the two cultivars were inoculated by viruliferous thrips (*Frankliniella occidentalis*) with inoculation access periods (IAPs) from 10 up to 2280 min. Higher transmission efficiencies and shorter median IAPs were found for the partially vector-resistant cv. 'Statesman'. Implications of the use of thrips resistant cultivars in tospovirus management programs are discussed.

*This chapter has been submitted for publication to Entomologia Experimentalis et Applicata as: van de Wetering, F., R. Veenstra, M. Y. Dutmer, R. Goldbach, and D. Peters. Adverse effects of vector resistance on tomato spotted wilt tospovirus infection in chrysanthemum.

Introduction

Tomato spotted wilt virus (TSWV) may cause major crop losses in chrysanthemum (Horst and Nelson, 1997). The main vector of TSWV (Wijkamp *et al.*, 1995) is currently the western flower thrips, *Frankliniella occidentalis* (Pergande). In Dutch greenhouses, this thrips species is even considered pest insect number one not only by causing (indirect) damage by transmitting TSWV, but due to its (direct) feeding damage, i.e. silvery scars and growth deformation, which results in drastically reduction of aesthetic quality of chrysanthemum plants and flowers.

To date, conventional control measures, including roguing of infected plants and thrips management, have not been completely effective to prevent tospovirus outbreaks (e.g. Daughtrey *et al.*, 1997; Stack *et al.*, 1997). Hence, alternative control measures, such as host plant resistance, need to be assessed for developing durable tospovirus control. So far, no cultivars completely resistant to thrips or TSWV have been identified, but several cultivars have been identified with various degrees of partial resistance to thrips. Previous studies revealed a low correlation between feeding scar production and TSWV incidence after long inoculation access periods (IAPs) (Allen *et al.*, 1990 and 1991; Broadbent and Allen, 1995; Broadbent *et al.*, 1990; Chapter 6), suggesting that thrips- resistant cultivars could not be used to control TSWV spread. Effects of a possible different feeding behaviour, and subsequent virus transmission, on thrips resistant and susceptible chrysanthemum plants will likely be eliminated in long incubation periods when they are forced to feed in order to survive. Short IAPs might better reflect the feeding behaviour of thrips on different chrysanthemum cultivars, and the actual situation in a chrysanthemum crop when thrips (can) rapidly move from one plant to another.

In this chapter, TSWV transmission to a partially vector-resistant and to a fully susceptible chrysanthemum cultivar was quantified using variable IAPs of up to two days.

Material and methods

Thrips and virus isolate

A *F. occidentalis* culture obtained from a greenhouse infestation on bean in the Netherlands (NL3) was used (Chapter 2). This culture was reared on *Phaseolus vulgaris* L. bean pods at 27 ± 0.5°C with a daily photoperiod of 16 h. The sex ratio of this culture was, on average, 1 male to 2 females. The TSWV isolate BR-01 (de Ávila *et al.*, 1990), used in all experiments, was maintained by thrips inoculation on *Datura stramonium* L. plants.

Chrysanthemum leaf disk assay

A chrysanthemum leaf disk assay was employed in these studies at 25° C, as described previously (Chapter 6). Five- to six-day old viruliferous adults were used to inoculate chrysanthemum leaf disks. Disks were cut from the middle leaves of the chrysanthemum cv. 'Statesman' and 'Sunny Cassa', which were partially resistant and susceptible to thrips feeding based feeding scar rates on leaves, respectively (van Dijken *et al.*, 1994). Differences between thrips partially resistant and susceptible chrysanthemum cultivars are most obvious in late autumn and winter (de Kogel *et al.*, 1997b), hence, experiments were executed in these seasons.

After IAPs of 48-h, the leaf disks were incubated on water for development of TSWV infection for two, three, four, five or nine days in a study to determine the period after which the chrysanthemum disks can optimally be analysed for the presence of virus in ELISA (Chapter 2). As control, disks were challenged by nonviruliferous thrips and incubated for the same periods on water. Leaf disks were denoted ELISA-positive when the ELISA readings were higher than the threshold value, determined as average value of the control + 3 times s.d. The percentage of ELISA-positive leaf disks and the average ELISA readings for the infected disks were calculated for the different incubation periods and chrysanthemum cultivars. An average of 30 leaf disks was used for each incubation period per chrysanthemum cultivar.

To quantify TSWV transmission, viruliferous adult thrips were given IAPs of 10, 20, 40, 80, 160, or 2280 min on leaf disks of the two chrysanthemum cultivars. A minimum IAP of 10 min was chosen, because not all thrips start to feed when shorter IAPs were used. After IAPs, the disks were incubated on water for either four or nine days and tested in ELISA to determine TSWV infection. An average of 44 and 22 adults were tested per IAP, for the 4-day and 9-day incubation period, respectively. The percentage of infected disks was plotted as a function of the IAPs. The IAP_{50}, expressing the time at which 50% of the viruliferous thrips did transmit, was estimated by log-probit analysis of transmission percentages (Sylvester, 1965). The IAP_{50} and its 90% fiducial limits were calculated by the method of Finney (1962). Data were analysed with the POLO-PC computer program (Anonymous, 1987).

Results

Incidence of TSWV infection in chrysanthemum cv. 'Statesman' and 'Sunny Cassa'

To quantify TSWV transmission to chrysanthemum, the leaf disk assay (see Chapter 6) was improved by determining the optimal leaf disk incubation period.

The minimal incubation period chosen (two days) resulted already in 52% and 10% infected disks of the chrysanthemum cv. 'Statesman' (partially resistant to thrips) and 'Sunny Cassa'(susceptible to thrips), respectively (Fig. 1).

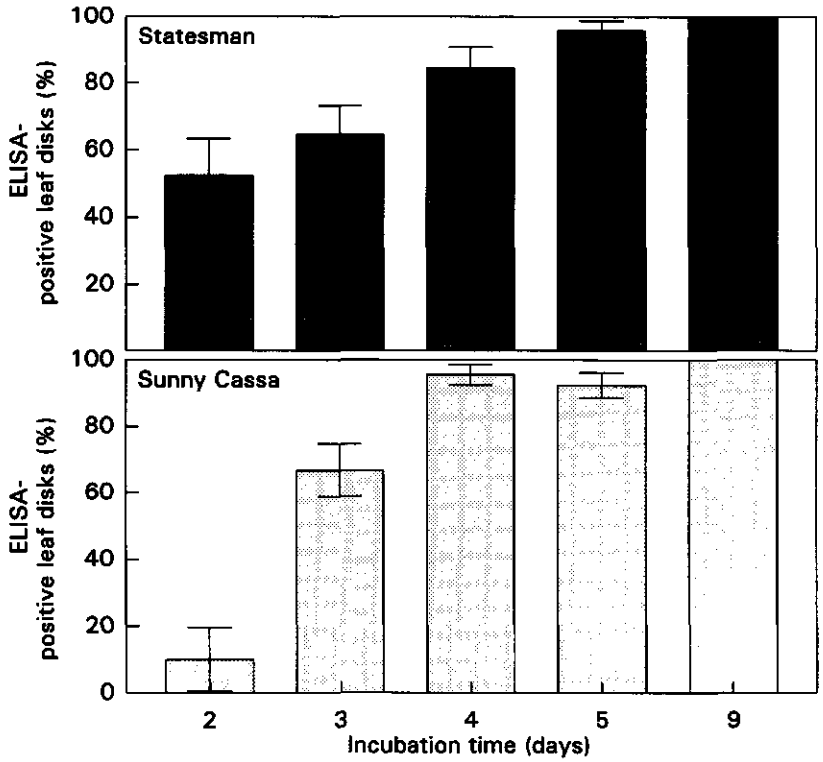

Figure 1. The percentage of enzyme-linked immunosorbent assay (ELISA)-positive leaf disks, after a 48 h inoculation access period by viruliferous *Frankliniella occidentalis* and subsequently incubated for different periods on water. The two chrysanthemum cv. 'Statesman' (thrips partially resistant) and 'Sunny Cassa' (thrips susceptible) were used. Leaf disks were denoted positive when the ELISA readings were higher than threshold value, which was determined as avg. value of noninfected plant material + 3 times s.d. Vertical bars indicate s.e.'s.

After four days, 84% of the 'Statesman' disks and 95% of the 'Sunny Cassa' disks were ELISA-positive, whereas all disks of both cultivars were infected after nine days of incubation. As the average ELISA readings of the disks increased with the length of incubation period (Fig. 2), TSWV was not detected in all infected disks when incubated for two and four days due viral antigen amounts that did not yet reach detectable levels in these disks (Fig. 2).

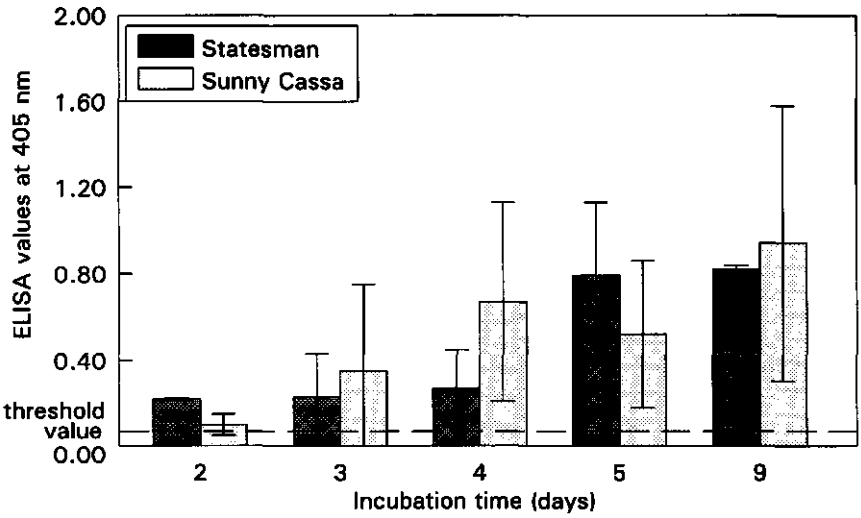

Figure 2. Enzyme-linked immunosorbent assay (ELISA) readings of ELISA-positive leaf disks, which were challenged by viruliferous *Frankliniella occidentalis* for 48 h and subsequently incubated for different periods on water. The two chrysanthemum cv. 'Statesman' (thrips partially resistant) and 'Sunny Cassa' (thrips susceptible) were used. Leaf disks were denoted positive when the ELISA readings were higher than the threshold value which was determined as avg. value of noninfected plant material + 3 times s.d. Vertical bars indicate s.e.'s.

To quantify TSWV transmission in further studies, disks were incubated for nine days, at which the maximal incidence of TSWV infection was found, and for four days, as after this incubation period the disks turned yellowish.

Quantification of TSWV transmission to chrysanthemum cv. 'Statesman' and 'Sunny Cassa'

The effect of (partially) vector resistance on TSWV transmission was quantified by determining the IAP_{50} on 'Statesman' or 'Sunny Cassa' disks, using IAPs varying in length from 10 min to two days.

When an IAP of 10 min was used, only 9% and 4% of 'Statesman' and 'Sunny Cassa' disks, respectively, were scored infected after four days of incubation (Fig. 3a).

These values increased with the lengths of IAPs. Significant differences in infection rates between 'Statesman' than for 'Sunny Cassa' disks were obtained, except for the longest IAP (2 days) as 78% ('Statesman') and 63.4% ('Sunny Cassa') disks were infected. The IAP_{50} was found to be 35.7 min for 'Statesman' and 102.9 min for 'Sunny Cassa' (Table 1), of which the 90% fiducial limits (FLs) of both cultivars did not overlap.

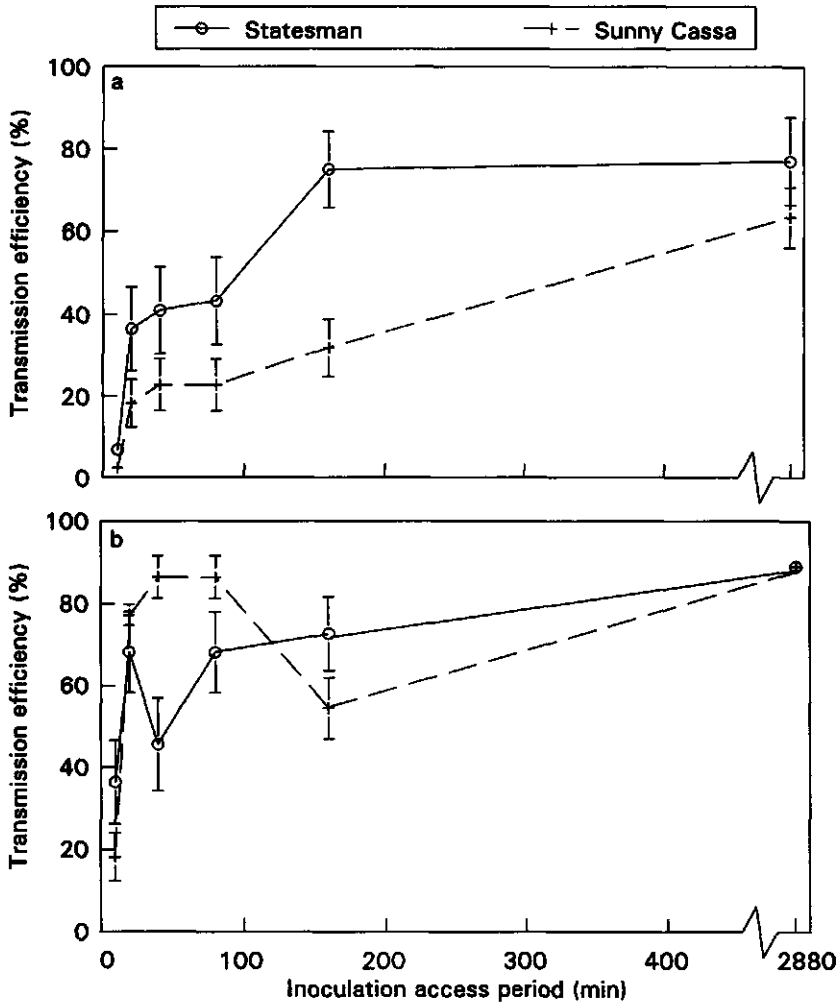

Figure 3. Tomato spotted wilt tospovirus transmission efficiency (± s.e.) by *Frankliniella occidentalis* as a function of the inoculation access period (IAP), after four (a) and nine (b) days of incubation of leaf disks on water for the development of virus infection. The IAP was either on the chrysanthemum cv. 'Statesman' (thrips partially resistant) or 'Sunny Cassa' (thrips susceptible). Vertical bars indicate s.e.'s.

Table 1. The median inoculation access period (IAP_{50}) of TSWV determined for a thrips partially resistant cv. 'Statesman' and a susceptible one 'Sunny Cassa', using chrysanthemum leaf disk assay, incubating the disks for either four or nine days.

Incubation time of leaf disks (days)	Cultivar	IAP_{50} (min)	90% fiducial limits (min)
4	Statesman	35.7	19-64
	Sunny Cassa	102.9	95-23
9	Statesman	9.6	ND[1]
	Sunny Cassa	13.5	ND[1]

[1] could not be determined.

Higher infection rates and shorter IAP_{50}s were scored when disks were incubated for nine instead of four days (Fig. 3a cf. Fig. 3b). After 10 min IAP, already 41% of 'Statesman' and 21% of 'Sunny Cassa' disks became infected, while with an IAP of 2 days, 89% infection was obtained for both cultivars (Fig. 3b). Differences in IAP_{50}s between 'Statesman' (9.6 min) and 'Sunny Cassa' (13.5 min) (Table 1) were still distinct (Table 1), although the 90% FLs could not be determined because of the IAP_{50}s were too close to the minimal IAP used. As these results obtained after nine days of incubation resemble those obtained after four days of incubation, both incubation periods can accurately be used to quantify tospovirus transmission to chrysanthemum which differ in their degree in vector resistance. From these studies it can be concluded that the partially (vector) resistant cv. 'Statesman' becomes more easily infected than the fully susceptible cv. 'Sunny Cassa'.

Discussion

In the present study, TSWV transmission to chrysanthemum cultivars, which differed in thrips susceptibility based on feeding damage rates, was quantified. Remarkably, for partially vector-resistant cv. 'Statesman' the IAP_{50} was shorter and infection efficiencies significantly higher (at almost all IAPs given) than for the fully susceptible cv. 'Sunny Cassa'. The higher infection efficiency for 'Statesman' may be explained as a result of a different feeding behaviour of thrips on this cultivar. On (partially) resistant cultivars, thrips may more frequently puncture a cell. These superficial punctures will leave the cell viable,

and support virus replication. More intensive feedings may take place on susceptible cultivars, leaving the cells destroyed, filled with air (silvery scar) and, hence, not capable to replicate virus.

The results obtained in this study, raises the question whether the use of vector-resistant plants adds to TSWV control. On one hand, our results indicate that primary TSWV infections are probably more easily established on thrips resistant than on susceptible chrysanthemum cultivars. On the other hand, the secondary spread (when TSWV is established in the crop) may be hampered by the lower survival rate of thrips on the vector-resistant cultivars (de Kogel *et al.*, 1997a) subsequently resulting in a lower number of viruliferous thrips. For some crops it is demonstrated that primary TSWV infections by dispersing thrips prevail over intercrop spread (Camann *et al.*, 1995; Gitiatis *et al.*, 1998; Laviña *et al.*, 1993; Todd *et al.*, 1996). If this would also hold for chrysanthemum, the use of thrips resistant cultivars is not the most effective way to control TSWV and may even have adverse effects.

Acknowledgements

This research was supported by fellowship, No. ABI22.2704, of the Foundation of Dutch Technology (STW) of the Netherlands Organisation for Scientific Research (STW). We thank Mr. S. van der Knaap of Fides Plants for the supply of chrysanthemum cuttings. We thank dr. C. Mollema from the Centre for Plant Breeding and Reproduction Research (CPRO-DLO), the Netherlands (CPRO-DLO) for useful discussions and his continuous interest in this study.

Chapter 8

Summary and concluding remarks

The introduction and rapid spread of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) in Western Europe since the 1980s led to a considerable increase of losses in different, mainly ornamental crops due to tomato spotted wilt tospovirus (TSWV) infections. Besides the losses inflicted by TSWV, *F. occidentalis* itself is also an important pest on many of these crops. Chrysanthemum is one of those crops which is affected by both TSWV and its vector. Breeding and selection of this plant species has resulted in the identification of a few chrysanthemum cultivars with some partial resistance to thrips. However, resistant cultivars to TSWV have not been successfully developed yet, meaning that the control of TSWV has to rely on other strategies. Besides sanitation programs, other successful strategies to control TSWV or *F. occidentalis* are currently not available. To develop durable and effective control measures and integrated pest management strategies, more detailed knowledge of the precise interactions between the virus, its vector and the threatened crop is required. Since transmission of TSWV is associated with ingestion of food, the feeding behaviour of thrips is one of the most determining factors in virus transmission. This study was therefore aimed to analyse the interactions between virus, vector and (chrysanthemum) plant in relation to thrips feeding.

Tospovirus is acquired by larvae and transmitted by old second instar larvae and adults after their emergence. As a first feature, the ability of larvae to acquire TSWV was analysed in relation to their age. The results obtained in a study with several *F. occidentalis* populations showed that the ability to acquire virus (defined as ingestion of virus by larvae, subsequently developing in viruliferous adults), dropped with the age of larvae (Chapter 2 and 3). A notable result was obtained with one of the populations (NL3), which could only acquire TSWV when larvae were in their first larval (L1) stage (Chapter 2).

Besides the age of the larvae at which they acquire virus, other parameters such as the *F. occidentalis* population involved, the feeding behaviour by the amount of food ingested, the virus species acquired and the host plant involved were studied for their effect on virus acquisition and transmission. Large and significant differences were found in TSWV transmission competencies between fourteen *F. occidentalis* populations which

originated from different countries all over the world. These differences were not affected by the amount of virus ingested or the host used as virus source. However, the use of another tospovirus species, impatiens necrotic spot virus (INSV), influenced the transmission differences between populations. The transmission efficiencies found appeared to be rather constant, supporting the view that the competence of a population to transmit TSWV is a stable, and, therefore, inherited property (Chapter 3).

The efficiency at which the *F. occidentalis* populations transmitted INSV was higher than that of TSWV. This observation confirms earlier reports that the different tospovirus species are transmitted at distinct rates by the same thrips population (Wijkamp *et al.*, 1995). It is likely that various isolates of TSWV will also be transmitted at different rates by one and the same *F. occidentalis* population as has been shown for some *Thrips tabaci* populations (Chatzivassiliou *et al.*, 1998). In contrast to TSWV, INSV is acquired by L1s as well as second larval instars (L2s) of the NL3 population (Chapter 3).

Further studies revealed that males of *F. occidentalis* are more efficient TSWV transmitters than females (Chapter 4). This feature was found for all fourteen *F. occidentalis* populations tested. The differences in virus transmission competencies between both sexes can be explained by differences in their feeding behaviour (Chapter 5). Males produce less silvery scars and make more frequent inoculation punctures than females. These punctures may represent the event during which the virus is successfully transmitted, as cells remain viable allowing virus to be replicated after virus-containing saliva injection. In addition, cells which are pierced and drained in the feeding process (resulting in silvery scars) are probably so destroyed that they will not support virus replication.

The different efficiencies by which males and females transmit virus may have an impact on the spread of the virus in a crop. Males may infect more plants than females as they show a higher mobility and the sex ratios in flights are male-biased. However, the contribution of males to the spread may be compensated or outweighed by the greater life expectation of females. Quantification of the development of an epidemic in terms of which part is caused by males and which by females, will be extremely difficult as the ratio between males and females will change continuously and their age can not accurately be determined.

To analyse whether thrips resistant chrysanthemum cultivars could effectively be used to control TSWV spread in this crop, fifteen cultivars were assessed for their susceptibility for this virus (Chapter 6). All cultivars were susceptible, irrespective their degree of thrips resistance. However, the number of plants that attracted an infection varied for each cultivar. It appeared in this study that the infection proceeds poorly in the infected plants and that the virus became unevenly distributed over the plant. A consequence of these observations is that in the chrysanthemum crop the virus will

disperse slowly from primary infected plants. No L1s emerging on such plants, or only a small proportion of them, will acquire virus. With the development of the infection in the plant and development of the thrips population, more larvae will be able to acquire virus and thus become transmitters. This means that the early developing population of thrips found on primary infected plants by incoming (dispersing) adults will hardly contribute to the (intercrop) spread of the virus an that, with time, the infection pressure may increase from these primary infected plants. Studies on assessing the development of viruliferous thrips in a population on a primary infected chrysanthemum plant should enhance our understanding of TSWV spread in chrysanthemum.

In most cases, tospovirus infections have been attributed to virus introduction from sources located outside the crop, and less to secondary (intercrop) spread in the crop as reported for groundnut, tomato and pepper (Camann *et al.*, 1995; Gitiatis *et al.*, 1998; Laviña *et al.*, 1993; Todd *et al.*, 1996). The incidence of TSWV infections in the Dutch chrysanthemum crops is low, despite the fact that TSWV transmission to chrysanthemum occurs highly efficiently (Chapter 6). Since the virus is not seed transmitted, the first infections may arise as a result from primary infections, subsequently followed by some secondary spread.

Using plants in testing the susceptibility of chrysanthemum for TSWV and vector resistance is a time- and labour-consuming activity. An expeditious leaf disk assay was introduced to assess this susceptibility (Chapter 6). This assay was also used to quantify TSWV transmission to a partially vector-resistant and a susceptible cultivar. It was shown that the inoculation access period in which 50% of the disks became infected (IAP_{50}) was shorter for a partially vector-resistant cultivar than for a susceptible cultivar, indicating that TSWV is more efficiently transmitted to the more vector resistant cultivar. This may be the result of a different feeding behaviour on the less preferred, partially resistant cultivar, resulting in a higher frequency of inoculation punctures in a unit of time, and subsequently in greater probability of successful transmission.

The spread of TSWV will certainly be affected by the attractiveness of the plant as food source of the thrips. The lower development rate of viruliferous thrips and the shorter life expectancy on partially vector-resistant cultivars likely reduces secondary spread. On the contrary, the mobility and dispersal of thrips and subsequent TSWV transmission in enhanced on partially resistant vector plants, and hence, the use of vector-resistant cultivars may not under all circumstances lead to an effective control of TSWV spread.

Literature cited

Adam, G, and H. Kegler, 1994. Tomato spotted wilt virus and related tospoviruses. Archiv für Phytopathologie and Planzenschutz 28: 483-504.

Alexandre, M. A. V., E. B. Rivas, L. M. L. Duarte, and M. H. Okuyama, 1996. Serological survey of tospovirus on chrysanthemum sp. crops in São Paulo State, Brazil. Fitopatologia Brasileira 21: 80-84.

Allen, W. R., and J. A. Matteoni, 1991. Petunia as indicator plant for use by growers to monitor for thrips carrying the tomato spotted wilt virus in greenhouses. Plant Disease 75: 78-82.

Allen, W. R., J. A. Matteoni, and A. B. Broadbent, 1990. Susceptibility of florist's chrysanthemum to tomato spotted wilt virus. Canadian Journal of Plant Pathology 12: 417-123.

Allen, W. R., J. A. Matteoni, and A. B. Broadbent, 1991. Factors relating to epidemiology and symptomatology in florist's chrysanthemum infected with tomato spotted wilt virus. In: H.-T. Hsu and R. H. Lawson (eds.), Proceedings of USDA Workshop, Virus-Thrips-Plant Interactions of Tomato Spotted Wilt Tospovirus, USDA ARS-87: 28-45.

Allen, W. R. , B. Tehrani, and R. Luft, 1993. Effect of horticultural oil, insecticidal soap, and film-forming products on western flower thrips and the tomato spotted wilt virus. Plant Disease 77: 915-918.

Ammar, E. D., 1994. Propagative transmission of plant and animal viruses by insects: Factors affecting vector specificity and competence. Advances in Disease Vector Research 10: 289-331.

Amin, P. W., D. V. R. Reddy, and A. M. Ghanekar, 1981. Transmission of tomato spotted wilt virus, causal agent of bud necrosis of peanut, by *Scirthothrips dorsalis* and *Frankliniella schultzei*. Plant Disease 65: 663-665.

Anonymous, 1979. Bureau of Sugar Experimental Station, 79th Annual Report: 32, 38.

Anonymous, 1987. POLO-PC. A users' guide to Probit Or LOgit analysis. LeOra Software Inc., Berkeley, CA: LeOra Software Inc., 20 pp.

Anonymous, 1989. EPPO data sheets on quarantine organisms – No. 177 – *Frankliniella occidentalis* (Pergande). OEPP/EPPO Bulletin 19: 725-731.

Anonymous, 1993. Distribution maps of pests - No.538 - *Frankliniella occidentalis*. CAB International Institute of Entomology, London.

Aramburu, J., J. Riudavets, J. Arnó, A. Laviña, and E. Moriones, 1997. The proportion of viruliferous individuals in field populations of *Frankliniella occidentalis*: implications for tomato spotted wilt virus epidemics in tomato. European Journal of Plant Pathology 103: 623-627.

de Ávila, A.C., P. de Haan, E.W. Kitajima, R. Kormelink, R. de O. Resende, R.W. Goldbach, and D. Peters, 1990. Serological differentiation of 20 isolates of tomato spotted wilt virus. Journal of General Virology 71: 2801-2807.

de Ávila, A. C., P. de Haan, E. W. Kitajima, R. Kormelink, R. de O. Resende, R. W. Goldbach, and D. Peters, 1992. Characterization of a distinct isolate of tomato spotted wilt virus (TSWV) from *Impatiens* sp. in the Netherlands. Journal of Phytopathology 134: 133-151.

Avrameas, S., 1969. Coupling of enzymes to proteins with glutaraldehyde. Immunochemistry 6: 43-52.

Bakker, F. M., and M. W. Sabelis, 1989. How larvae of *Thrips tabaci* reduce attack success of phytoseiid predators. Entomologia Experimentalis et Applicata 50: 47-51.

Bandla, M. D., L. R. Campbell, D. E. Ullman, and J. L. Sherwood, 1998. Interaction of tomato spotted wilt tospovirus (TSWV) glycoproteins with a thrips midgut protein, a potential cellular receptor for TSWV. Phytopathology 88: 98-104.

Bandla, M. D., D. M. Westcot, D. E. Ullman, and J. L. Sherwood, 1995. Thrips midgut proteins that bind to tomato spotted wilt virus glycoproteins. Acta Horticulturae 431: 341-349.

Bartlett, A. C., and N. J. Gawell, 1993. Determining whitefly species. Science 261:1333-1334.

Bedford, I. D., R. W. Briddon, J. K. Brown, R. C,. Rosell, and P. G. Markham, 1994. Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. Annals of Applied Biology 125: 311-325.

Bedford, I. D., R. W. Briddon, P. Jones, N. Alkaff, and P. G. Markham, 1992. *Bemisia tabaci* - Biotype characterisation and the threat of this whitefly species to agriculture. BCPC - Pests and Diseases 3: 1235-1240.

Bedford, I. D., R. W. Briddon, P. G. Markham, J. K. Brown, and R. C. Rosell, 1993. A new species of *Bemisia* or biotype of *Bemisia tabaci* (Genn.) as a future pest of European agriculture. Plant Health and the European Single Market, BCPC Monograph 54: 381-386.

Bergh, J. C., and J.-P. Le Blanc, 1997. Performance of western flower thrips (Thysanoptera: Thripidae) on cultivars of miniature rose. Journal of Economic Entomology 90: 679-688.

Berlinger, M. J., S. Lebuish-Mordechi, D. Fridja, and N. Mor, 1993. The effects of types of greenhouse screens on the presence of western flower thrips: a preliminairy study. International Organisation of Biological Control/ West Palaearctic Regional Section Bulletin 16: 13-16.

Black, L.L., H. A. Hobbs, and J. M. Gatti, 1991. Tomato spotted wilt virus resistance in *Capsicum chinense* PI 152225 and PI 159236. Plant Disease 75: 863.

Bournier, A., 1983. Les Thrips. Biologie, Importance Agronomique. INRA, Paris, 128 pp.

Brittlebank, C.C., 1919. Tomato diseases. Journal of Agriculture, Victoria17: 231-235.

Broadbent, A. B., and W. R. Allen, 1995. Interactions within the western flower thrips/tomato spotted wilt virus/host plant complex on virus epidemiology. In: B. L. Parker, M. Skinner, and T. Lewis (eds.), Thrips Biology and Management, New York, Plenum Press: 193-196.

Broadbent, A. B., J. A. Matteoni, and W. R. Allen, 1990. Feeding preferences of the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), and incidence of tomato spotted wilt virus among cultivars of florist's chrysanthemum. Canadian Entomologist 122: 1111-1117.

Brodbeck, B, J. Funderburk, J. Stavisky, P. Andersen, and S. Olson, 1998. Cultural tactics for management of western flower thrips and tospovirus. In: D. Peters and R. Goldbach (eds.), Recent Progress in Tospoviruses and Thrips Research, Wageningen, the Netherlands: 68-70.

Brødsgaard, H. F., 1989. *Frankliniella occidentalis* (Thysanoptera: Thripidae) – a new pest in Danish glasshouses. A review. Tiddskrift foer Planteavl 93: 83-91.

Brødsgaard, H. F., 1993a. Coloured sticky traps for thrips monitoring on glasshouse cucumbers. International Organisation of Biological Control/ West Palaearctic Regional Section Bulletin 16: 19-22.

Brødsgaard, H. F., 1993b. Monitoring thrips in glasshouse pot plant crops by means of blue sticky traps. International Organisation of Biological Control/ West Palaearctic Regional Section Bulletin 16: 29-32.

Brødsgaard, H. F., 1994a. Effect of photoperiod on the bionomics of *Frankliniella occidentalis* (Pergande) (Thysanoptera; Thripidae). Journal of Applied Biology 117: 498-507.

Brødsgaard H. F., 1994b. Insecticide resistance in European and African strains of western flower thrips (Thysanoptera: Thripidae) tested in a new residue-on-glass test. Journal of Economic Entomology 87: 1141-1146.

Brown, J. K., S. A. Coats, I. D. Bedford, P. G. Markham, and J. Bird, 1992. Biotypical characterisation of *Bemisia tabaci* populations based on esterase profiles, DNA fingerprinting, virus transmission and bioassays to key host plant species. Phytopathology 82:1104.

Brown, S., J. Todd, and A. Culbreath, 1996. Effects of selected cultural practices on the incidence of tomato spotted wilt virus in peanuts. Acta Horticulturae 431: 491-198.

Brown, S., J. Todd, A. Culbreath, D. Gorbett, F. Shokes, J. Baldwin, and J. Beasley, 1998. An integrated management strategy for tomato spotted wilt of peanut in Georgia. In: D. Peters and R. Goldbach (eds.), Recent Progress in Tospoviruses and Thrips Research, Wageningen, the Netherlands: 75-76.

Byrne, F. J., and A. L. Devonshire, 1993. Insensitive acetylcholinesterase and esterase polymorfism in susceptible and resistant populations of the tobacco whitefly *Bemisia tabaci* (Genn.). Pesticide Physiology 45: 34-42.

Camann, M. A., A. K. Culbreath, J. Pickering, J. W. Todd, and J. W. Demski, 1995. Spatial and temporal patterns of tomato spotted wilt epidemics in peanut. Phytopathology 85: 879-885.

Campbell, B. C., J. E. Duffus, and P. Baumann, 1993. Determining whitefly species. Science 261:1333.

Chatzivassiliou, E. K., N. I. Katis, and D. Peters, 1998. Transmission of tomato spotted witl virus (TSWV) by *Thrips tabaci* grown on tobacco or non-tobacco hosts. In: D. Peters and R. Goldbach (eds.), Recent Progress in Tospoviruses and Thrips Research, Wageningen, the Netherlands: 59-62.

Chen, C. C., and R. J. Chiu, 1996. A tospovirus infecting peanut in Taiwan. Acta Horticulturae 431: 57-67.

Chisholm, I.F., and T. Lewis, 1984. A new look at thrips (Thysanoptera) mouthparts, their action and effects of feeding on plant tissue. Bulletin of Entomological Research 74: 663-675.

Cho, J. J., D. M. Custer, S. H. Brommonschenkel, and S. D. Tanksley, 1996. Conventional breeding: host plant resistance and the use of molecular markers to develop resistance to tomato spotted wilt virus in vegetables. Acta Horticulturae 431: 367-378.

Cho, J. J., R. F. L. Mau, T. L. German, R. W. Hartmann, L. S. Yudin, D. Gonsalves, and R. Provvidenti, 1989. A multidisciplinary approach to management of tomato spotted wilt virus in Hawaii. Plant Disease 73: 375-383.

Clark, M. F., and A. N. Adams, 1977. Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. Journal of General Virology 34: 475-483.

Cook, D. F., B. J. Houlding, E. C. Steiner, D. C. Hardie, and A. C. Postle, 1996. The native anthocorid bug (*Orius armatus*) as a field predator of *Frankliniella occidentalis* in Western Australia. Acta Horticulturae 431: 507-512.

Cortês, I., J. Livieratos, A. Derks, D. Peters, and R. Kormelink, 1998. Molecular and serological characterization of iris yellow spot virus, a new and distinct tospovirus species. Phytopathology: 1276-1282.

Cortês, I., A. Pereira, R. Goldbach, D. Peters, and R. Kormelink, 1998. An RT-PCR procedure to amplify S RNA sequences of distinct tospoviruses. In: D. Peters and R. Goldbach (eds.), Recent Progress in Tospoviruses and Thrips Research, Wageningen, the Netherlands: 35-37.

Dal Bó, E., L. Ronco, A. M. Alippi, and R. Fernández, 1995. Tomato spotted wilt virus on chrysanthemum in Argentina. Plant Disease 79: 538.

Daniels, J., A. A. Husain, P. B. Hutchinson, and C. A. Wismer, 1969. An insectary method for testing sugar cane varieties for resistance to Fiji Disease. Proceedings International Society of Sugar Cane Technology 13: 1100-1106.

Daughtrey, M. L., R. K. Jones, J. W. Moyer, M. E. Daub, and J. R. Baker, 1997. Tospoviruses strike the greenhouse industry, INSV has become a major pathogen on flower crops. Plant Disease 81: 1220-1230.

DeAngelis, J. D., D. M. Sether, and P. A. Rossignol, 1993. Survival, development, and reproduction in western flower thrips (Thysanoptera: Thripidae) exposed to impatiens necrotic spot virus. Environmental Entomology 22: 1308-1312.

Derks, A. F. L. M., and M. E. C. Lemmers, 1996. Detection of tospoviruses in bulbous crops and their transmissibility by vegetative propagation. Acta Horticulturae 432: 132-139.

Dewey, R. L. Semorile, O. Gracia, and O. Grau, 1993. TSWV N protein gene sequence comparison among an Argentine isolate and other tospoviruses. Abstract of the IXth International Congress of Virology, Glasgow, Scotland, Page Bros Norwich: 357.

van Dijken, F. R., M. T. A. Dik, B. Gebala, J. de Jong, and C. Mollema, 1994. Western flower thrips (Thysanoptera: Thripidae) effect on chrysanthemum cultivars: plant growth and leaf scarring in nonflowering plants. Journal of Economic Entomology 87: 1312-1317.

Egan, B. T., Ryan, C. C., and Francki, R. I. B. 1989. Fiji Disease. In: C. Ricaud, B. T. Egan, A. G. Gillaspie, and C. G. Hughes (eds.), Diseases of Sugarcane. Major Diseases. Elsevier, Amsterdam, the Netherlands: 263-287.

Finney, D. J., 1962. Probit analysis, a statistical treatment of the sigmoid response curve. Cambridge University Press, 318 pp.

Francki, R. I. B., C. C. Ryan, T. Hatta, J. Rohozinski, and C. J. Grivell, 1986. Serological detection of Fiji disease virus antigens in the planthopper *Perkinsiella saccharida* and its inefficient ability to transmit the virus. Plant Pathology 35: 324-328.

Fujisawa, I., K. Tanaka, and M. Ishii, 1988. Tomato spotted wilt virus transmissibility by three species of thrips, *Thrips setosus*, *Thrips tabaci* and *Thrips palmi*. (In Japanese) (Abstract) Annual Phytopathological Society of Japan 54: 392.

Funderburk, J., J. Stavisky, C. Tipping, and S. Olson, 1998. Reevaluating the role of natural enemies and regulation of thrips populations. In: D. Peters and R. Goldbach (eds.), Recent Progress in Tospoviruses and Thrips Research, Wageningen, the Netherlands: 71-74.

Gardner, M. W., C. M. Tompkins, and O. C. Whipple, 1935. Spotted wilt of truck crops and ornamental plants. Phytopathology 25: 17.

Gaston, K. J., and L. A. Mound, 1993. Taxonomy, hypothesis testing and the biodiversity crisis. Proceedings of the Royal Society, London 251: 139-142.

Gera, A., J. Cohen, R. Salomon, and B. Raccah, 1998a. Irish yellow spot tospovirus detected in onion (*Allium cepa*) in Israel. Plant Disease 82: 127.

Gera, A., A. Kritzman, J. Cohen, and B. Raccah, 1998b. Tospoviruses infecting bulb crops in Israel. In: D. Peters and R. Goldbach (eds.), Recent Progress in Tospoviruses and Thrips Research, Wageningen, the Netherlands: 86-87.

Gerin, C. Th. Hance, and G. van Impe, 1994. Demographical parameters of *Frankliniella occidentalis* (Pergande) (Thysanoptera, Thripidae). Journal of Applied Entomology 118: 370-377.

German, T. L., D. E. Ullman, and J. W. Moyer, 1992. Tospoviruses: Diagnosis, molecular biology, phylogeny, and vector relationships. Annual Review of Phytopathology 30: 315-348.

Gildow, F. E., and S. M. Gray, 1993. The aphid salivary gland basal lamina as a selective barrier associated with vector-specific transmission of barley yellow dwarf luteoviruses. Phytopathology 83: 1293-1302.

Gitaitis, R. D., C. C. Dowler, and R. B. Chalfant, 1998. Epidemiology of tomato spotted wilt in pepper and tomato in Southern Georgia. Plant Disease 82: 752-756.

Granval de Millan, N., R. Piccolo, and O. Gracia, 1998. Potato (*Solanum tuberosum* L.) tuber transmission of tospoviruses (TSWV,GRSV and TCSV) in Mendoza province, Argentina. In: D. Peters and R. Goldbach (eds.), Recent Progress in Tospoviruses and Thrips Research, Wageningen, the Netherlands: 46-47.

Greenhough, D. R., and L. L. Black, 1990. Aluminum-surfaced mulch: an approach to the control of tomato spotted wilt virus in solanaceous crops. Plant Disease 74: 805-808.

Goldbach, R., and G. Kuo, 1996. Introduction. Acta Horticulturae 431: 21-28.

Goldbach, R., and D. Peters, 1994. Possible causes of the emergence of tospovirus diseases. Seminars of Virology 5: 113-120.

Guldemond, J. A., and E. den Belder, 1993. Supervised control in chrysanthemums: one years experience. International Organisation of Biological Control/ West Palaearctic Regional Section Bulletin 16: 51-54.

de Haan, P., 1991. Exploring and exploiting the RNA genome of tomato spotted wilt virus. PhD Dissertation Agricultural University Wageningen, the Netherlands, 135 pp.

de Haan, P., T. Ultzen, M. Prins, J. Gielen, R. Goldbach, and M. van Grinsven, 1996. Transgenic tomato hybrids resistant to tomato spotted wilt virus infection. Acta Horticulturae 431: 417-426.

Halbert, S. E., B. J. Connelly, G. W. Bishop, and J. L. Blackmer, 1992. Transmission of barley yellow dwarf virus by field collected aphids (Homoptera: Aphididae) and their relative importance in barley yellow dwarf epidemiology in southwestern Idaho. Annals of Applied Biology 121: 105-121.

Hampton, R., E. Ball, and S. de Boer, 1990. Serological methods for detection and identification of viral and bacterial plant pathogens. The American Phytopathological Society, Minnesota, USA, 389 pp.

Harrewijn, P., W.F. Tjallingii, and C. Mollema, 1996. Electrical penetration by western flower thrips. Entomologia Experimentalis et Applicata 79: 345-353.

Hausbeck, M. K., R. A. Welliver, M. A. Derr, and F. E. Gildow, 1992. Tomato spotted wilt virus survey among greenhouse ornamentals in Pennsylvania. Plant Disease 76: 795-800.

Helyer, N. L. , and P. J. Brobyn, 1992. Chemical control of western flower thrips (*Frankliniella occidentalis* Pergande). Annals of Applied Biology 121: 219-231.

van den Heuvel, J. F. M., and D. Peters, 1989. Improved detection of potato leafroll virus in plant material and in aphids. Phytopathology 79:963-967.

Higgins, C. J., and J. H. Myers, 1992. Sex ratio patterns and population dynamics of western flower thrips (Thysanoptera: Thripidae). Environmental Entomology 21: 322-330.

Hirao, J., and H. Inoue, 1978. Transmission efficiency of rice waika virus by the green rice leafhoppers, *Nephotettix* spp. (Hemiptera: Cicadellidae). Applied Entomology and Zoology 13: 264-273.

Hirao, J., and H. Inoue, 1979. Transmission characteristics of rice waika virus by the green rice leafhoppers, *Nephotettix cincticeps* UHLER (Hemiptera: Cicadellidae). Applied Entomology Zoology 14: 44-50.

Horst, R. K., and P. E. Nelson, 1997, Compendium of Chrysanthemum Diseases, APS Press, USA: 37-38.

Hsu, H., and R. H. Lawson, 1991. Detection of tomato spotted wilt virus by enzyme-linked immusorbent assay, dot-blot assay and direct tissue blotting. In: H. Hsu, and R. H. Lawson (eds.), Proceedings of USDA Workshop, Virus-Thrips-Plant Interactions of Tomato Spotted Wilt Virus, USDA ARS-87: 120-126.

Hunter, W. A., and D. E. Ullman, 1989. Analysis of mouthpart movement during feeding of *Frankliniella occidentalis* (Pergande) and *F. schultzei* Trybom (Thysanoptera: Thripidae). International Journal of Insect Morphology and Embryology 18: 161-171.

Iwaki, M., Y. Honda, K. Hanada, H. Tochihara, T. Yonaha, K. Hokama, and T. Yokoyama, 1984. Silver mottle disease of watermelon caused by tomato spotted wilt virus. Plant Disease 79: 572-576.

Jacobson, R. J., 1997. Integrated pest management (IPM) in glasshouses. In: T. Lewis (ed.), Thrips as Crop Pests, CAB International: 639-666.

de Jager, C. M., R. P. T. Butôt, and J. A. Guldemond, 1995. Genetic variation in chrysanthemum for resistance to western flower thrips and *Thrips tabaci*. In: B. L. Parker, M. Skinner, and T. Lewis (eds.), Thrips Biology and Management, Plenum Press, New York: 403-406.

Jain, R., H. Pappu, S. Pappu, M. Krishnareddy, and A. Vani, 1998. Evidence that the watermelon bud necrosis tospovirus from India is a distinct virus species belonging to serogroup IV. Wageningen, the Netherlands: 48.

Jarošik, V., and Lapchin, L., 1998. Development time of western flower thrips, *Frankliniella occidentalis*: consequences in breeding for host plant resistance. Plant Protection Service 34: 116-119.

Kikkert, M., C. Meurs, F. van de Wetering, S. Dorfmüller , D. Peters, R. Kormelink, and R. Goldbach, 1998. Binding of tomato spotted wilt virus to a 94-kDa thrips protein. Phytopathology 88: 63-69.

Kring, J. B., and D. J. Schuster, 1992. Management of insects on pepper and tomato with UV-reflective mulches. Florida Entolomologist 75: 119-129.

de Kogel, W. J., 1997. Host plant resistance to western flower thrips: variable insects and plants. PhD thesis, University of Amsterdam, the Netherlands, 126 pp.

de Kogel, W. J., M. van der Hoek, M. T. A. Dik, F. R. van Dijken, and C. Mollema, 1997a. Variation in performance of western flower thrips populations on a susceptible and a partially resistant chrysanthemum cultivar. In: W. J. de Kogel, Host Plant Resistance To Western Flower Thrips: Variable Insects and Plants. PhD thesis, University of Amsterdam, the Netherlands: 85-94.

de Kogel, W. J., M. van der Hoek, M. T. A. Dik, B. Gebala, F. R. van Dijken, and C. Mollema, 1997b. Seasonal variance in resistance of chrysanthemum cultivars to *Frankliniella occidentalis*. Euphytica 97: 283-288.

de Kogel, W. J., M. van der Hoek, and C. Mollema, 1997c. Variation in performance of western flower thrips populations on susceptible and partially resistant cucumber. Entomologia Experimentalis et Applicata 83: 73-80.

Kormelink, R., P. de Haan, C. Meurs, D. Peters, and R. Goldbach, 1992. The nucleotide sequence of the M RNA segment of tomato spotted wilt virus, a bunyavirus with two ambisense RNA segments. Journal of General Virology 73: 2795- 2804.

Kormelink, R., E. W. Kitajima, P. de Haan, D. Zuidema, D. Peters, and R. Goldbach, 1991. The nonstructural protein (NS_s) encoded by the ambisense S RNA segment of tomato spotted wilt virus is associated with fibrous structures in infected plant cells. Virology 181: 459-468.

Kormelink, R., M. Storms, J. van Lent, D. Peters, and R. Goldbach, 1994. Expression and subcellular location of the NS_m protein of tomato spotted wilt virus (TSWV), a putative movement protein. Virology 200: 56-65.

Lakshmi, K. V., J. A. Wightman, D. V. R. Reddy, G. V. Ranga Rao, A. A. M. Buiel, and D. D. R. Reddy, 1995. Transmission of peanut bud necrosis virus by *Thrips palmi* in India. In: B. L. Parker, M. Skinner, and T. Lewis (eds.), Thrips Biology and Management, Plenum Press, New York: 179-184.

Laviña, A., J. Aramburu, J. Riudavets, J. Arnó, and E. Moriones, 1993. Epidemiology of TSWV on tomato in northeast Spain. Abstract of the IXth International Congress of Virology, Glasgow, Scotland, Page Bros Norwich: 137.

Law, M. D., and J. W. Moyer, 1990. A tomato spotted wilt-like virus with aserologically distinct N protein. Journal of General Virology 71: 933-938.

Lewis, T., 1973. Thrips, their Biology, Ecology, and Economic Importance. Academic Press, London, 349 pp.

Louro, D. 1996. Detection and identification of tomato spotted wilt virus and Impatiens necrotic spot virus in Portugal. Acta Horticulturae 431: 99-105.

Ludwig, G. V., B. M. Christensen, T. M Yuill, and K. T. Schultz, 1989. Enzyme processing of La Crosse virus glycoprotein G1: a bunyavirus-vector infection model. Virology 171: 108-113.

Ludwig, G. V., B. A. Israel, B. M. Christensen, T. M. Yuill, and K. T. Schultz, 1991. Role of La Crosse virus glycoproteins in attachment of virus to host cells. Virology 181: 564-571.

Malchau, W. 1990. Die präimaginale Entwicklung von *Frankliniella tenuicornis* (UZEL, 1895) (*Thysanoptera*). Entomologische Nachrichten und Berichten 4: 129-134.

Mantel, N. A., and M. van de Vrie, 1988. De Californische trips, *Frankliniella occidentalis,* een nieuwe schadelijke tripssoort in de tuinbouw onder glas in Nederland. Entomologische Berichten 48: 140-144.

Marchoux, G., K. Gebreselassie, and M. Villevieille, 1991. Detection of tomato spotted wilt virus and transmission by *Frankliniella occidentalis* in France. Plant Pathology 40: 347-351.

Matteoni, J. A., and W. R. Allen, 1989. Symptomatology of tomato spotted wilt virus infection in florist' chrysanthemum. Canadian Journal of Plant Pathology 11: 373-380.

McGrath, P. F., and B. D. Harrison, 1995. Transmission of tomato leaf curl geminiviruses by *Bemisia tabaci*: effects of virus isolate and biotype. Annals of Applied Biology 126: 307-316.

Mollema, C., F. van Dijken, K. Reinink, and R. Jansen, 1992. An automatic and accurate evaluation of thrips-damage. Image-analysis: a new tool in breeding for resistance. In: S. B. J. Menken, J. H. Visser, and P. Harrewijn (eds.), Proceedings of the 8th International Symposium on Insect-Plant Relationships. Dordrecht, Kluwer Academic Publishers: 261-263.

Mollema, C., M. M. Steenhuis, H. Inggamer, and C. Soria, 1993. Evaluating the resistance to *F. occidentalis* in cucumber: genetic variation and effects upon thrips biology. International Organisation of Biological Control/ West Palaearctic Regional Section Bulletin 16: 77-82.

Mollema, C., G. Steenhuis, and P. van Rijn, 1990. Development of a method to test resistance to Western flower thrips (*Frankliniella occidentalis*) in cucumber. Bulletin of International Organisation of Biological Control 13: 113-116.

Moulton, D., 1931. Western *Thysanoptera* of economic importance. Journal of Economic Entomology 24: 1031-1036.

Mound, L. A., 1996. The Thysanoptera vector species of tospoviruses. Acta Horticulturae 431: 299-309.

Mound, L. A., and G. Kibby, 1998. Thysanoptera: and identifcation guide. CAB International, 70 pp.

Mungomery, R. W., and A. F. Bell, 1939. Fiji disease and its transmission. Bureau of Sugar Experimental Station, Division Pathology, Bulletin 17: 50-51.

Murphy, G. D., and A. B. Broadbent, 1993. Development and implementation of IPM in greenhouse floriculture in Ontario, Canada. International Organisation of Biological Control/ West Palaearctic Regional Section Bulletin 16: 119-122.

Murphy, F. A., C. M. Fauquet, and D. H. L. Bishop, 1995. Virus taxonomy: classification and nomenclature of viruses: sixth report of the International Committee on Taxonomy of Viruses, Virology Division, International Union of Microbial Societies. Wien, Springler Verlag, 586 pp.

Nagata, T., A. K. Inoue-Nagata, R. Goldbach, and D. Peters. Study of primary infection of TSW in thrips midgut using a novel immuno-staining method. In: D. Peters and R. Goldbach (eds.), Recent Progress in Tospoviruses and Thrips Research, Wageningen, the Netherlands: 54-55.

Nagata, T., A. K. Inoue-Nagata, H. M. Smid, R. Goldbach, and D. Peters. Tissue tropism is related to vector competence of *Frankliniella occidentalis* for tomato spotted wilt tospovirus. Journal of General Virology 80: 507-515.

Noordam, 1943. Over het voorkomen van 'spotted wilt' in Nederland. Tijdschrift over Plantenziekten 49: 117-119.

Ochoa, M., D. L., E. Zavaleta-Mejía, R. M. Johansen N., A. Herrera G., and E. Cárdenas Soriano, 1996a. Tospoviruses, weeds and thrips associated with chrysanthemum (*Dendranthemum grandiflora* cv. Polaris). International Journal of Pest Management 42: 157-159.

Ochoa, M., D. L., E. Zavaleta-Mejía, R. M. Johansen N., A. Herrera G., and E. Cárdenas Soriano, 1996b. Effect of using certified virus-free cuttings, mulching and floating covers on chrysanthmeum production (*Dendranthemum grandiflora* cv. Polaris) and incidence of tospoviruses. International Journal of Pest Management 42: 161-164.

Ohnishi, J., D. Hosokawa, I. Fujisawa, and S. Tsuda, 1998. Tomato spotted wilt tospovirus movement into salivary glands during pupation of thrips vector, *Thrips setosus*, is associated with its transmissibility. In: D. Peters and R. Goldbach (eds.), Recent Progress in Tospoviruses and Thrips Research, Wageningen, the Netherlands: 51-53.

Palmer, J. M., D. V. R. Reddy, J. A. Wightman, and G. V. Ranga Rao, 1990. New information on the thrips vectors of tomato spotted wilt virus in groundnut crops in India. International Arachis Newsletter 7: 24-25.

Pang, S. Z., P. Nagpala, M. Wang, J. Slightom, and D. Gonsalves, 1992. Resistance to heterologous isolates of tomato spotted wilt virus in transgenic tobacco expressing its nucleocapsid protein gene. Phytopathology 82: 1223-1229.

Parrella, M. P., and V. P. Jones, 1987. Development of integrated pest management strategies in floricultural crops. Bulletin of the Entomological Society of America 33: 28-34.

Parrella, M. P. and T. Lewis, 1997. Integrated pest management (IPM) in field crops. In: T. Lewis (ed.), Thrips as Crop Pests, CAB International: 595-614.

Parker, B. L., M. Skinner, M. Brownbridge, A. Adamowicz, and F. C. Su, 1996. Entomopathogenic fungi for thrips management: recent advances. Acta Horticulturae 431: 521-534.

Perring, T. M., A. D. Cooper, R. J. Rodriguez, C. A. Farrar, and T. S. Bellows, 1993a. Identification of a whitefly species by genomic and behavioral studies. Science 259: 74-77.

Perring, T. M., C. A. Farrar, A. D. Cooper, and T. S. Bellows, 1993b. Determining whitefly species. Science 261: 1334-1335.

Peters, D., 1998. An updated list of plant species susceptible to tospoviruses. In: D. Peters and R. Goldbach (eds.), Recent Progress in Tospoviruses and Thrips Research, Wageningen, the Netherlands: 107-110.

Peters, D., I. Wijkamp, F. van de Wetering, and R. Goldbach, 1996. Vector relations in the emergence and epidemiology of tospoviruses. Acta Horticulturae 431: 29-43.

Pittman, H. A., 1927. Spotted wilt of tomatoes. Preliminary note concerning the transmission of the "spotted wilt" of tomatoes by an insect vector (*Thrips tabaci* Lind.). Commonwealth of Australia, Council of Science and Industrial Research Bulletin 1: 74-77.

de Ponti, O. M. B., 1982. Plant resistance to insects: a challenge to plant breeders and entomologists. In: Proceedings, Fifth International Symposium on Insect-Plant Relationships. Center for Agricultural Publishing and Documentation, Wageningen, the Netherlands: 337-347.

Prado, E., and W. F. Tjallingii, 1994. Aphid activities during sieve element punctures. Entomologia Experimentalis et Applicata 72: 157-165.

Prins, M., P. de Haan, R. Luyten, M. van Veller, M. Q. J. M. van Grinsven, and R. Goldbach, 1995. Broad resistance to tospoviruses in transgenic tobacco plants expressing three tospoviral nucleoprotein gene sequences. Molecular Plant-Microbe Interactions 8: 85-91.

Reddy, D. V. R., M. R. Sudarshana, A. S. Ratna, A. S. Reddy, P. W. Amin, I. K. Kumar, and A. K. Murthy, 1990. The occurrence of yellow spot virus, a member of tomato spotted wilt group, on peanut (*Arachis hypogaea* L.). In: H.-T. Hsu, and Lawson, R.H. (eds.), Virus-Thrips-Plant Interactions of TSWV, Proceedings of the USDA Workshop, Beltsville, USA. National Technical Service, Springfield, USA: 77-78.

van Rensburg, G. D. J., and J. H. Giliomee, 1990. A comparison of females and males of *Cicadulina anestae* and *C. mbila* (Homoptera: Cicadellidae) as vectors of maize streak virus. Phytophylactica 22: 241-243.

Resende, R. de O., De Ávila, A. C., Goldbach, R. W., and Peters, D. 1991. Comparison of polyclonal antisera in the detection of tomato spotted wilt virus using double antibody sandwich and cocktail ELISA. Journal of Phytopathology 132:46-56.

Resende, R. de O., L. Posser,T. Nagata, I. C. Bezerra, M. I. Lima, E. W. Kitajima, and A. C. de Àvila, 1996. New tospoviruses found in Brazil. Acta Horticulturae 431: 78-89.

van Rijn, P. C. J., C. Mollema, and G. M. Steenhuis-Broers, 1995. Comparative life history studies of *Frankliniella occidentalis* and *Thrips tabaci* (Thysanoptera: Thripidae) on cucumber. Bulletin of Entomological Research 85: 285-297.

Rijpkema, J. J. M., 1993. Statistiek met Statgraphics. Academic Service, Schoonhoven, 271 pp.

Riley, D., H. Pappu, and D. Adams, 1998. Use of multiple tactics for the reduction of thrips and tomato spotted wilt virus in tomatoes in Georgia. In: D. Peters and R. Goldbach (eds.), Recent Progress in Tospoviruses and Thrips Research, Wageningen, the Netherlands: 88.

Robb, K. L., J. Newman, J. K. Virzi, and M. P. Parella, 1995. Insecticide resistance in the western flower thrips. In: B. L. Parker, M. Skinner, and T. Lewis (eds.), Thrips Biology and Management, Plenum Press, New York: 341-346.

Robb, K., and M. Parrella, 1989. An integrated approach to preventing western flower thrips and TSWV in the greenhouse. GrowerTalks 53 : 26-32.

Rodriguez, C. M. , L. V. Madden, L. R. Nault, and R. Louie, 1993. Spread of maize chlorotic dwarf virus from infected corn and johnsongrass by *Graminella nigrifons*. Plant Disease 77: 55-60.

Roosjen, M., and H. Cevat, 1995. Actuele verschuivingen in ziekten en plagen. Vakblad voor de Bloemisterij 29: 29-34.

Sakimura, K. 1962. The present status of thrips-borne viruses. In: K. Maramorosch (ed.), Biological Transmission of Disease Agents, Academic Press, New York: 33-40.

Sakimura, K., 1963. *Frankliniella fusca*, an additional vector of tomato spotted wilt virus, with notes on *Thrips tabaci*, another vector. Phytopathology 53: 412-415.

Samuel, G., J. G. Bald, and H. A. Pittman, 1930. Investigations on "spotted wilt" of tomatoes. Commonwealth of Australia, Council Scien. Ind. Res. Bulletin 44, 64 pp.

Satyanarayana, T., S. E. Mitchell, D. V. R. Reddy, S. Brown, S. Kresovich, R. Jarret, R. A. Naidu, and J. W. Demski, 1996. Peanut bud necrosis tospovirus S RNA: complete nucleotide sequence, genome organization and homology to other tospoviruses. Archives of Virology 141: 85-98.

Saxena, R. C., and A. A. Barrion, 1987. Biotypes of insect pests of agricultural crops. Insect Science and Applications 8: 453-458.

Schulman, R. S., 1992. Statistics in Plain English with Computer Applications. Chapman and Hall, New York, 483 pp.

Scott, S. J., P. J. McLeod, F. W. Montgomery, and C. A. Hander, 1989. Influence of reflective mulch on incidence of thrips (Thysanoptera: Thripidae: Phlaeothripidae) in staked tomatoes. Journal of Entomological Science 4: 422-427.

Sherman, J. M., J. W. Moyer, and M. E. Daub, 1996. Genetically engineered resistance to tomato spotted wilt virus in chrysanthemum (*Dendranthema grandiflora*): a model system for virus protection in ornamental crops. Acta Horticulturae 431: 432-441.

Singh, S. J., and M. Krishanareddy, 1996. Watermelon bud necrosis: a new tospovirus disease. Acta Horticulturae 431: 68-77.

Soria, C., and C. Mollema, 1995. Life-history parameters of western flower thrips on susceptible and resistant cucumber genotypes. Entomologia Experimentalis et Applicata 74: 177-184.

Stack, P. A., L. B. Stack, and F. A. Drummond, 1997. A two-year grower survey of thrips and *Tospovirus* incidence and management in Maine greenhouses. Journal of Agricultural Entomology 14: 205-224.

Stevens, J. M., 1964. Tomato breeding. Project RPT. W-VVI, Dept. Agr. Tech. Ser., South Africa.

Stevens, M. R., S. J. Scott, and R. C. Gergerich, 1992. Inheritance of a gene for resistance to tomato spotted wilt virus (TSWV) from *Lycopersicon peruvianum* Mill. Euphytica 59: 9-17.

Storms, M., 1998. The role of NS_m during tomato spotted wilt virus infection. PhD Dissertation Agricultural University Wageningen, the Netherlands, 128 pp.

Sylvester, E. S., 1965. The latent period of pea-enation mosaic virus in the pea aphid, *Acyrthosiphon pisum* (Harris) – an approach to its estimation. Virology 56: 62-67.

Sylvester, E. S., 1980. Circulative and propagative virus transmission by aphids. Annual Review of Entomology 25: 257-286.

Tashiro, H., 1967. Selfwatering acrylic cages for confining insects and mites and detached leaves. Journal of Economic Entomology 60: 354-356.

Tavella, L., A. Alma, A. Conti, and A. Arzone, 1996. Evaluation of the effectiveness of *Orius* spp. in controlling *Frankliniella occidentalis*. Acta Horticulturae 431: 499-506.

Terry, L. I., and C. K. Kelly, 1993. Patterns of change in secondary and tertiary sex ratios of the Terebrantian thrips, *Frankliniella occidentalis*. Entomologia Experimentalis et Applicata 66: 213-225.

Thompson, G. J., and J. J. B. van Zijl, 1996. Control of tomato spotted wilt virus in tomatoes in South Africa. Acta Horticulturae 431: 379-392.

Todd, J. W., A. K. Culbreath, and S. L. Brown, 1996. Dynamics of vector populations and progress of tomato spotted wilt disease relative to insecticide use in peanuts. Acta Horticulturae 431: 483-490.

Tommasini, M.G., and S. Maini, 1995. *Frankliniella occidentalis* and other thrips harmful to vegetable and ornamental crops in Europe. In: J.C. van Lenteren, A.J.M. Loomans, M.G. Tommasini, S. Maini and J. Riudavets (eds.), Biological Control of Thrips Pests, Wageningen Agricultural University Papers 95-1, Veenman Drukkers, the Netherlands: 3-42.

Trichilo, P. J., and T. F. Leigh, 1988. Influence of resource quality on the reproductive fitness of flower thrips (Thysanoptera: Thripidae). Annals of Entomological society of America 81: 64-70.

Tsuda, S., I. Fujisawa, J. Ohnishi, D. Hosokawa, and K. Tomaru, 1996. Localization of tomato spotted wilt tospovirus in larvae and pupae of the insect vector *Thrips setosus*. Phytopathology 86: 1199-1203.

Ullman, D. E., J. J. Cho, R. F. L. Mau, D. M. Westcot, and D. M. Custer, 1992. A midgut barrier to tomato spotted wilt virus acquisition by adult western flower thrips. Phytopathology 82: 1333-1342.

Ullman, D. E., T. L. German, J. L. Sherwood, D. M. Westcot, and F. A. Cantone, 1993. Tospovirus replication in insect vector cells: Immuno-cytochemical evidence that the non-structural protein encoded by the S RNA of tomato spotted wilt tospovirus is present in thrips vector cells. Phytopathology 83: 456-463.

Ullman, D. E., D. M. Westcott, K. D. Chenault, J. L. Sherwood, T. L. German, M. D. Bandla, F. A. Cantone, and H. L. Duer, 1995. Compartmentalization, intracellular transport, and autophagy of tomato spotted wilt tospovirus proteins in infected thrips cells. Phytopathology 85: 644-654.

Vaira, A. M., P. Roggero, E. Luisoni, V. Masenga, R. G. Milne, and V. Lisa, 1993. Characterization of two tospoviruses in Italy: tomato spotted wilt virus and impatiens necrotic spot virus. Plant Pathology 42: 530-542.

Verhoeven, J. Th. J., and H. N. Cevat, 1996. Nieuw virus bedreigt de chrysantenteelt. Vakblad voor de Bloemisterij 3: 48-49.

Verhoeven, J. Th. J., and J. W. Roenhorst, 1994. Tomato spotted wilt virus: ecological aspects in ornamental crops in the Netherlands from 1989 up to 1991. Acta Horticulturae 377: 175-182.

Verhoeven, J. Th. J., and J. W. Roenhorst, 1998. Occurrence of tospoviruses in the Netherlands. In: D. Peters and R. Goldbach (eds.), Recent Progress in Tospoviruses and Thrips Research, Wageningen, the Netherlands: 77-80.

Verhoeven, J. Th. J., J. W. Roenhorst, I. Cortês, and D. Peters, 1996. Detection of a novel tospovirus in chrysanthemum. Acta Horticulturae 432: 44-52.

Vijayalakshmi, K., 1994. Transmission and ecology of *Thrips palmi* Karny, the vector of peanut bud necrosis virus. Ph.D. thesis, Andrha Pradesh Agricultural University, Hyderabad, 99 pp.

Webb, S., J. Tsai and F. Mitchell, 1998. Bionomics of *Frankliniella bispinosa* and its transmission of tomato spotted wilt virus. In: D. Peters and R. Goldbach (eds.), Recent Progress in Tospoviruses and Thrips Research, Wageningen, the Netherlands: 67.

van de Wetering, F., R. Goldbach, and D. Peters, 1996a. TSWV is transmitted by *Frankliniella occidentalis* only after acquisition during the first larval stage. Acta Horticulturae 431: 350-358.

Wijkamp, I., N. Almarza, R. Goldbach, and D. Peters, 1995. Distinct levels of specificity in thrips transmission of tospoviruses. Phytopathology 85: 1069-1074.

Wijkamp, I., R. Goldbach, and D. Peters, 1996a. Differential susceptibilities between leaf disks and plants in the transmission of tomato spotted wilt virus by *Frankliniella occidentalis* to TSWV hosts and transgenic plants. Journal of Phytopathology 144: 355-362.

Wijkamp, I., R. Goldbach, and D. Peters, 1996b. Virus-vector interactions of tomato spotted wilt virus in *Frankliniella occidentalis* is neither accompanied by pathological effects nor by transovarial transmission. Entomologia Experimentalis et Applicata 81: 285-292.

Wijkamp, I., J. van Lent, R. Kormelink, R. Goldbach, and D. Peters, 1993. Multiplication of tomato spotted wilt virus in its insect vector, *Frankliniella occidentalis*. Journal of General Virology 74: 341-349.

Wijkamp, I., and D. Peters, 1993. Determination of the median latent period of two tospoviruses in *Frankliniella occidentalis*, using a novel leaf disk assay. Phytopathology 83: 986-991.

Wijkamp, I., F. van de Wetering, R. Goldbach, and D. Peters, 1996c. Transmission of tomato spotted wilt virus by *Frankliniella occidentalis*; median acquisition access and inoculation access period. Annals of Applied Biology 129: 303-313.

Yeh, S.-D., and T. F. Chang, 1995. Nucleotide sequence of the N gene of watermelon silver mottle virus, a proposed new member of the genus Tospovirus. Phytopathology 85: 58-64.

Yeh, S.-D., Y.-C. Lin, Y.-H. Cheng, C.-L. Jih, M.-J. Chen, and C.-C. Chen,1992. Identification of tomato spotted wilt-like virus on watermelon in Taiwan. Plant Disease 76: 835-840.

Yeh, S.-D., Y.-C. Peng, C.-H. Chao, and C.-C. Chen, 1998. Peanut chlorotic fan-spot virus is serologically and phylogenetically distinct from other tospoviruses. In: D. Peters and R. Goldbach (eds.), Recent Progress in Tospoviruses and Thrips Research, Wageningen, the Netherlands: 42-44.

Yudin, L. S., J. J. Cho, and W. C. Mitchell, 1986. Host range of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), with special reference to *Leucaena glauca*. Environmental Entomology 15: 1292-1295.

Zhao G., W. Liu, J. M. Brown, and C. O. Knowles, 1995. Insecticide resistance in field and laboratory strains of western flower thrips (Thysanoptera: Thripidae). Journal of Economic Entomology 88: 1164-1170.

van Zijl, J. J. B., S. E. Bosch, and C. P. J. Coetzee, 1986. Breeding tomatoes for processing in South Africa. Acta Horticulturae 431: 69-74.

Zur Strassen, R., 1986. *Frankliniella occidentalis* (Pergande, 1895) from North America as a new Thysanopterous inhabitant of European greenhouses (German, English abstract). Nachrichtenbl. Deut. Pflantzenschutzd. 38: 86-88.

Samenvatting

Het tomatenbronsvlekkenvirus, in het Engels 'tomato spotted wilt tospovirus (TSWV)', is een virus dat tot de economisch meest schadelijke plantenvirussen van de wereld behoort. Het virus maakt deel uit het genus *Tospovirus*, en wordt van de ene naar de andere plant overgedragen door tripsen (1 à 2 mm lange insecten). Van de 5000 tot nu toe beschreven tripsensoorten is van slechts zeven vastgesteld dat zij tospovirussen kunnen overdragen. De meest efficiënte overdrager (vector) van TSWV is de californische trips, *Frankliniella occidentalis* (Pergande). Door de introductie en snelle verspreiding van deze tripsensoort over het westen van Europa sinds 1980, is er sprake van een aanzienlijke toename van TSWV schade in een groot aantal gewassen, zoals tomaat, sla, en chrysant. Behalve dat *F. occidentalis* zeer efficiënt TSWV kan overdragen, veroorzaakt dit insect zelf ook aanzienlijke schade door vraat (zilverachtige vlekken op bloemen en bladeren, en groeimisvorming). In Nederland is deze tripsensoort momenteel zelfs het meest schadelijke insect in kasteelten.

Een gewas dat van zowel TSWV als trips schade ondervindt is de chrysant. Door middel van veredeling en selectie is een aantal chrysantencultivars geïdentificeerd met een zekere mate van afweer (resistentie) tegen trips. Echter, er zijn nog geen cultivars beschikbaar die resistentie tegen TSWV bezitten. De bescherming van chrysant tegen TSWV infecties is daarom gebaseerd op andere middelen, zoals het virus- en trips-vrij houden van de kassen. Deze voorzorgsmaatregelen kunnen echter niet altijd voorkomen dat een chrysantenteelt besmet raakt met TSWV, zodat grote opbrengstderving kan optreden door lagere productie, cosmetische schade en zelfs door afsterven van het gewas. Om teelten van chrysanten effectief en duurzaam tegen TSWV te beschermen, is het noodzakelijk om alternatieve en/of additionele strategieën voor preventie en bestrijding te ontwikkelen. Gedetailleerde kennis over hoe TSWV door tripsen in chrysant wordt verspreid is daarvoor essentieel. Derhalve zijn gedurende dit promotieonderzoek de interacties tussen virus, trips, en chrysant bestudeerd waarbij vooral de relatie tussen voedingsgedrag van de trips en overdracht van TSWV centraal stond.

Allereerst wordt in hoofdstuk 1 een overzicht gegeven van de eigenschappen van het virus (TSWV), de trips vector *(F. occidentalis)*, en van de huidige strategieën om TSWV in chrysant te bestrijden.

De verspreiding van TSWV door trips wordt gekenmerkt door overdracht door oude tweede stadium (L2) larven en de volwassen dieren (adulten), waarbij het virus verworven dient te worden in het larvale stadium. In hoofdstuk 2 is onderzocht in welk stadium de larven het virus kunnen verwerven. Het bleek dat van de Nederlandse *F. occidentalis*

populatie NL3 alleen eerste stadium (L1) larven in staat waren TSWV te verwerven. Binnen dit stadium nam het vermogen om virus te verwerven echter af naarmate de larven ouder werden. Aangezien van andere getoetste *F. occidentalis* populaties de L2's eveneens in staat waren TSWV te verwerven, en dat een ander tospovirus, impatiens necrotic spot virus (INSV), bovendien door zowel L1's als L2's van de NL3 populatie kon worden verworven (hoofdstuk 3), toont aan dat de exclusieve TSWV verwerving door L1's een specifiek kenmerk van de NL3 populatie is. Verder bleek dat de efficiëntie van virusoverdracht een stabiele, karakteristieke eigenschap is voor elk van de veertien getoetste *F. occidentalis* populaties, afkomstig van verschillende delen uit de wereld.

De efficiëntie van TSWV overdracht voor mannelijke en vrouwelijke tripsen is vergeleken in hoofdstuk 4. Voor alle veertien *F. occidentalis* populaties bleek dat mannelijke tripsen het virus efficiënter overdragen dan de vrouwelijke individuen. Voor beide geslachten gold dat de virusoverdracht afnam bij toenemende leeftijd, simultaan met een afnemende voedselconsumptie. Verder is in hoofdstuk 5 aangetoond dat het verschil in efficiëntie van virusoverdracht tussen mannelijke en vrouwelijke tripsen samenhangt met een verschillend voedingsgedrag. Het is aannemelijk gemaakt dat succesvolle overdracht geschiedt via het slechts kortstondig aanprikken van een plantencel door de trips. Bij intensief voedingsgedrag, resulterend in 'zilverschade', zal een trips een plantencel volledig leegzuigen, en heeft het virus dientengevolge geen kans zich te vermenigvuldigen en de plant te infecteren. Uit de behaalde resultaten blijkt dat door een hogere mobiliteit en een lagere frequentie aan intensieve voedingen, mannelijke tripsen enerzijds minder zilverschade veroorzaken maar anderzijds juist meer effectieve virusinoculaties teweeg brengen.

Vervolgens is onderzocht of het gebruik van trips-resistente planten, waarop tripsen onder andere een lage ontwikkelingssnelheid hebben en weinig zilverschade veroorzaken, mogelijk als middel gebruikt kan worden om TSWV infecties in chrysant te minimaliseren. Daarvoor is eerst de gevoeligheid van vijftien commercieel gebruikte cultivars voor TSWV onderzocht (hoofdstuk 6). Alle getoetste cultivars bleken gevoelig voor TSWV infectie, onafhankelijk van hun resistentie tegen trips. Tevens bleek dat de cultivars zeer makkelijk door trips konden worden geïnfecteerd met TSWV; de kans op succesvolle overdracht was meer dan 50% wanneer één virus-overdragende trips op een chrysantenplant werd gezet. Om in de toekomst de virusgevoeligheid van chrysantencultivars in zeer korte tijd en met beperkte inspanning vast te kunnen stellen werd een bladponsentoets ontwikkeld. Tevens werd deze toets in hoofdstuk 7 gebruikt om TSWV overdracht naar vatbare- en (gedeeltelijke)-trips resistente chrysantencultivars te kwantificeren. Het bleek dat TSWV makkelijker (efficiënter) wordt overgedragen naar de partieel trips-resistente cultivar dan naar de vatbare, waarschijnlijk door meer effectieve virusinoculaties op deze minder aantrekkelijke cultivar voor trips. Het gebruik van trips-resistente planten zal daarom enerzijds resulteren in een verminderde ontwikkeling van een trips populatie, en daardoor

minder virus-overdragende tripsen, maar anderzijds resulteren in een efficiëntere TSWV overdracht van virusoverdragende tripsen naar deze planten. Derhalve zal de inzet van trips-resistente cultivars niet onder alle omstandigheden leiden tot een inperking van TSWV infecties in de chrysantenteelt.

Nawoord

En dan is het nu tijd voor het nawoord. Een leuk stuk om te schrijven omdat het waarschijnlijk veel gelezen gaat worden (zoals bij alle proefschriften) en omdat ik op deze plek alle mensen kan bedanken die me de afgelopen jaren gesteund hebben, dus eh...BEDANKT. Door deze steun heb ik met veel plezier aan mijn promotieonderzoek gewerkt en heb het binnen de gestelde periode afgerond met dit proefschrift. Terugkijkend op die vierenhalf jaar realiseer ik me ik heel veel geleerd heb en dat er ontzettend veel veranderd is in mijn leven. Tjonge, wat is het allemaal snel gegaan!

Een aantal mensen wil ik apart in dit nawoord noemen, en dan wil ik beginnen met mijn directe begeleiders Rob Goldbach en Dick Peters. Ik wil hun bedanken voor hun kritische kijk op het schrijven van dit proefschrift, maar vooral voor de vrijheid die ze me gegeven hebben om het onderzoek in te vullen. Zo heb ik zowel fundamenteel als praktijkgericht onderzoek kunnen doen, heb 'onderzoek-uitstapjes' naar -onder andere- de DLO instituten 'Centrum voor Plantenveredelings en Reproduktieonderzoek' (CPRO) en 'Instituut voor Planteziektenkundig Onderzoek' (IPO) gemaakt, en heb mijn behaalde resultaten gepresenteerd op vele (inter)nationale congressen.

Mijn promotieonderzoek maakte deel uit van een groot project, wat geïnitieerd en gecoördineerd werd door mijn twee andere begeleiders Chris Mollema en Steph Menken. Hun enthousiasme voor dit onderzoek, het regelen van samenkomsten met de andere (ex-) projectgangers Willem Jan de Kogel, Egbert de Vries, Mous Sabelis, Hans Breeuwer, Leo van der Geest en Folchert van Dijken, met of zonder de gebruikerscommissie, heeft geleid tot nuttige discussies, bedankt daarvoor. Marieke van der Hoek, ook een projectganger, verdient een extra bedankje omdat zij de (vele) tripsenpopulaties op CPRO-DLO in stand gehouden heeft en met mij vele MEGAproeven tot een goed einde gebracht.

De meeste proeven zijn uitgevoerd op het Laboratorium voor Virologie, in het 'trips-lab'. At times, I had to transfer thrips for eight hours a day (BORING!). You can imagine how happy I was when someone from the 'thrips-group' joined me so we could chat and gossip, therefore a 'thank you' to Ineke Wijkamp, Tatsuya Nagata, Giuseppe Stancanelli, Elizabeth Chatzivassilou, Marisa Romero and dr. Meir Klein. Natuurlijk heb ik het werk niet alleen gedaan, de ex-studenten Jan Hulshof, Roel Veenstra, Rolf Bolt, Karin Posthuma, Marjolein Dutmer, Marjoleine Weerdesteyn, en Daniel Lüdeking hebben me erg goed geholpen. Het was leuk om met jullie samen te werken! Ook wil ik Ine Derksen bedanken voor het voederen van de tripsen en schoonmaken van de potten, en Janneke Saayer voor het steeds maar weer verstrekken van goed antisera. Een bedankje is ook op zijn plaats

voor de mensen van Unifarm (kassen), fotolocatie, en technische dienst. Zonder hen zou het werk een stuk minder soepel verlopen zijn.

Naast de 'trips-groep' was er nog een groep waarin ik 'hoorde': de TSWV groep. Alice, Axel, Claire, Cor, Danny, Erwin, Frank, Isabel, Marc, Marcel, Richard, Rolf en vele (gast)medewerkers, ik heb veel opgestoken van jullie -vooral moleculaire- werk dat besproken werd tijdens de wekelijkse discussies op donderdag en in de wandelgang(en). Maar nog meer wil ik jullie, en alle andere mensen van het lab, bedanken voor het gezellig met elkaar omgaan, in goede, slechte, late, vroege, en verre tijden. Een aparte vermelding is er voor Marjolein, mijn kamergenote en nu ook paranimf. Het was leuk dat het zo klikte: er was niet alleen tijd om serieus te werken, er was veel tijd om te lachen maar vooral om te praten over van alles en nog wat. Gezellig, en ik hoop dat we contact blijven houden!

Naast werk is er natuurlijk ook een privé-leven. Na een dag (hard) werken was het lekker om de stress (enzo) even van me af te zetten tijdens het hockeyen. Meiden bedankt dat ik mijn verhaal, lief en leed, altijd bij jullie kwijt kon. Ik hoop dat dit in de toekomst zo zal blijven. De komende jaren even geen zwangerschappen meer, dus nu weer gezellig een volledig jaar hockeyen.

Ook wil ik papa, mama, Alieke en de rest van de (schoon)familie in dit nawoord noemen (ook al willen jullie dit waarschijnlijk niet). Ondanks dat dit promotieonderzoek niet helemaal jullie pakkie an was (begrepen jullie het nu echt?), bleven jullie belangstelling hebben voor mijn werk en me door dik en dun steunen, bedankt.

En dan wil ik dit nawoord afsluiten met een stukje voor mijn beste vriend, tevens mijn 'husband' en de papa, René. Ik wil je bedanken voor al die nachten dat je me begeleid hebt naar het lab (eng hoor zo alleen in het lab), voor je begrip ("waar zijn de tripsen nu ik zie ze niet!") en voor je steun -in het bijzonder tijdens het schrijven van dit proefschrift-, maar vooral voor de ontzettend leuke en gelukkige tijd die we eerst met z'n 2-en en nu met z'n 4-en hadden en hebben. Hopelijk kunnen we privé en werk zo goed blijven combineren.

Fennet

Curriculum vitae

Fennet van de Wetering werd geboren op 13 september 1970 te Delft. Zij behaalde in 1989 het diploma O. V. W. O. aan het Berlingh College te Beverwijk en begon in datzelfde jaar aan de studie Plantenziektenkunde aan de Landbouwuniversiteit te Wageningen. Na vierenhalf jaar behaalde zij haar ingenieurstitel met een afstudeeropdracht en een praktijktijd in de Virologie en Entomologie. Gedurende haar praktijktijd in Costa Rica heeft zij zich gericht op de taxonomie van tripsen bij het 'Museo de Insectos' onder leiding van dr. Paul Hanson, en heeft zij de aanwezigheid van tospovirussen in Costa Rica onderzocht bij 'Centro de Investigation en Biologia Celular y Molecular' onder leiding van dr. Carmen Rivera. Aansluitend aan haar studie trad zij in april 1994 in dienst van de Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO) als onderzoeker in opleiding, en werd onder verantwoordelijkheid van de Stichting voor de Technische Wetenschappen (STW) gedetacheerd bij het Laboratorium voor Virologie van de Landbouwuniversiteit Wageningen. Het promotieonderzoek richtte zich op de variabiliteit in het aanpassingsvermogen van de californische trips met betrekking tot overdracht van het tomatenbronsvlekkenvirus (TSWV). Zij heeft, binnen haar aanstellingsperiode van ruim vier jaar, de resultaten van het onderzoek beschreven in dit proefschrift.

Publicatielijst

Peters. D., I. Wijkamp, F. van de Wetering, and R. Goldbach, 1995. Dynamics of the spread of tospoviurses by their vectors. In: A. A. M. Buiel, J. E. Parlevliet, and J. M. Lenné (eds.), Recent Studies on Peanut Bud Necrosis Disease: Proceedings of a Meeting 20 Mar 1995, ICRISAT, Asia Center: 17-24.

Peters, D., I. Wijkamp, F. van de Wetering, and R. Goldbach, 1996. Vector relations in the emergence and epidemiology of tospoviruses. Acta Horticulturae 431: 29-43.

van de Wetering, F., R. Goldbach, and D. Peters, 1996. Transmission of tomato spotted wilt virus by *Frankliniella occidentalis* after viral acquisition during the first larval stage. Acta Horticulturae 431: 350-358.

van de Wetering, F., R. Goldbach, and D. Peters. 1996. Transmission of tomato spotted wilt virus by *Frankliniella occidentalis* is a prerequisite for transmission. Phytopathology 86: 900-905.

van de Wetering, F., J. Hulshof, F. R. van Dijken, R. Goldbach, and D. Peters, 1996. Differences in virus transmission and scar production among males and females of the western flower thrips. In: Proceedings of the Seventh Meeting of Experimental and Applied Entomologists of the Netherlands Entomological Society 7:183-190.

Kikkert, M., C. Meurs, F. van de Wetering, S. Dorfmüller, D. Peters, R. Kormelink, and R. Goldbach. 1997. Binding of tomato spotted wilt virus to a 94-kDa thrips protein. Phytopathology 88: 63-69.

Peters, D., A. Loomans, T. Nagata, I. Wijkamp, and F. van de Wetering, 1997. Methods to rear and maintain thrips in tospovirus transmission studies. In: Proceedings of Invertebrates in Capacity Conference, 1997, Tuscon, USA: 152-159.

van de Wetering, F., R. Veenstra, M. Dutmer, K. Posthuma, M. van der Hoek, R. Goldbach, and D. Peters, 1997. Tomato spotted wilt virus transmission correlated with trhips susceptibility in chrysanthemum, using a leaf disk assay. In: Proceedings of the Eighth Meeting of Experimental and Applied Entomologists of the Netherlands Entomological Society 8:127-132.

Wijkamp, I., F. van de Wetering, R. Goldbach, and D. Peters, 1997. Transmission of tomato spotted wilt virus by *Frankliniella occidentalis*; median acquisition and inoculation access period. Annals of Applied Biology 129:303-313.

van de Wetering, F., J. Hulshof, K. Posthuma, P. Harrewijn, R. Goldbach, and D. Peters, 1998. Distinct feeding behavior between sexes of *Frankliniella occidentalis* results in higher scar production and lower tospovirus transmission by females. Entomologia Experimentalis et Applicata 88: 9-15.

Storms, M., F. van de Wetering, T. Nagata, J. van Lent, and R. Goldbach. Expression of the movement protein of tomato spotted wilt virus in its vector *Frankliniella occidentalis.* Submitted to Virus Research.

van de Wetering, F., M. van der Hoek, R. Goldbach, C. Mollema, and D. Peters. Variation in tospovirus transmission efficiencies among *Frankliniella occidentalis* populations. Submitted to Bulletin of Entomological Research.

van de Wetering, F., M. van der Hoek, R. Goldbach, and D. Peters. Differences in tomato spotted wilt virus vector competency among males and females of *Frankliniella occidentalis*. Submitted to Entomologia Experimentalis et Applicata.

van de Wetering, F., K. Posthuma, R. Goldbach, and D. Peters. Assessment of susceptibility of different chrysanthemum cultivars for tomato spotted wilt virus susceptibility in chrysanthemum. Accepted in Plant Pathology.

van de Wetering, F., R. Veenstra, M. Y. Dutmer, R. Goldbach, and D. Peters. Adverse effect of vector resistance on tomato spotted wilt virus infection in chrysanthemum. Submitted to Entomologia Experimentalis et Applicata.